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TUBERCULOSIS ERADICATION CONFERENCE

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U. S. DEPARTMENT OF AGRICULTURE
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FOREWORD

This publication contains the formal presentations given at the Tuberculosis Eradication Conference sponsored by the Agricultural Research Service, Animal Disease Eradication Division at the School of Veterinary Medicine, Kansas State University, August 17 - 21, 1959.

Many of these papers are authoritative reports of professional opinions expressed by outstanding persons on the subject of tuberculosis.

As is true of the published proceedings of the previous similar Tuberculosis Eradication Conference held in 1958 at Michigan State University, the compilation of these papers into one report provides a comprehensive reference of pertinent information about tuberculosis. Some of these papers are complements of those published from the Michigan Conference. All of them will help to provide a better understanding of the total problem of tuberculosis and more effective methods to achieve eradication of this disease.

Attendance at this conference was mainly made up of State livestock officials and those responsible for conducting tuberculosis eradication work within the several States.

A panel of these State officials which summarized the work of the conference went on record attesting to the value of the proceedings and favoring a continuation of such educational efforts so that the goal of tuberculosis eradication may be achieved.

R. J. Anderson
Director, Animal Disease
Eradication Division

Issued December 1960

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ASSISTANCE OF EXTENSION SERVICE IN ANIMAL DISEASE ERADICATION PROGRAMS

William G. Amstein, State Leader, Agricultural Specialists
Kansas State University of Agriculture and
Applied Science, Manhattan

May I join with the others in welcoming you to Kansas State University. We are all pleased to have you on the campus.

Here at Kansas State University of Agriculture and Applied Science, and at every other Land-Grant headquarters for Agricultural Extension work, I think you will find a sincere and genuine interest in your program. We desire to cooperate so that you can achieve as rapidly as practical these animal disease eradication programs. Education is basic to any successful program. This certainly applies to animal disease control programs. Likewise, full cooperation is essential if a good control program is achieved. All interested areas must be tied in and associated if a successful program is developed.

The Extension Service at Federal, State, and county levels is able to assist in the education phases of any recognized animal disease eradication program. We operate at all three levels. It is our responsibility to participate in the educational phases of these programs.

In terms of Extension Veterinarians, we do not have a large group in the United States. In fact, their assistance is available in only about 20 States with a total of 30 men. I hope that each State may soon have one or more extension specialists in this field of veterinary medicine.

We relate to the Federal Service through Mr. Charles E. Bell of the Federal Extension Service, who is in the Animal Industry Branch of the Division of Agricultural Programs. He coordinates and correlates our animal disease programs through your Federal counterparts in the Animal Disease Eradication Division of the Agricultural Research Service.

To handle the many programs at the State level, we have, along with the School of Veterinary Medicine staff, a strong core of educational workers in the Director of the Extension Service; District Agents and Specialists in Veterinary Medicine, Animal Production (Livestock, Dairy, and Poultry); and other supporting subject-matter groups.

However, our real strength is at the county level. Our county Extension Agents and their local organization(s) will do much to provide success to any program. In fact, none of our educational programs are successful without their total support.

In most counties, our County Extension Agents have organized livestock committees of various types. Given a job assignment, these committee members (who usually represent every township or community area) are in a position, with good leadership, to do much of the local

organizational and educational work required for a successful animal disease eradication program.

As valuable as these committees are, perhaps even greater strength is available through the other family members. I refer to the wife and children of these committee members, who through membership in Home Economics, 4-H, and FFA groups and other organizations can bring a great deal of interest and impetus to any worthwhile educational program. These groups are well organized and have an active membership in most communities.

Here in Kansas, our Extension Veterinarian, Dr. Melvin Osburn, and Home Economics Health Specialist, Martha Brill, have cooperated in several educational health programs often involving both animal and human diseases. This total support of home economics, 4-H, agricultural (livestock), and other groups is essential to the early success of any educational program.

In the Extension Service of Kansas State University, and of most States, there are two supporting groups that are also vital to any successful educational program. I refer to the Information and the Radio and Television groups. No matter how well informed we may be and "sold" on any particular program, the job is not and will not be completed successfully until the story has been told and retold many times.

Regulatory work usually goes only as fast as the educational process that accompanies and precedes it. Here at Kansas State and everywhere else, I think you can depend upon a total educational approach. With it, success can be achieved. We are all willing to do our part.

STATE-FEDERAL TEAMWORK

Charles Figy, Assistant to the Secretary, USDA
Washington, D. C.

You will notice the subject assigned to me is "State-Federal Teamwork." There is much to be said on this subject. The State and Federal agencies must work together, and I believe most can be accomplished if we have full cooperation and by mutal agreement each agency is willing to assume its share of the cost--rather than for one agency to tell the others what they must do.

We have demonstrated this by the policy statements we have worked out in an effort to more clearly define the lines of responsibility.

You will remember that a few years ago an effort was made to reduce Federal funds for livestock disease eradication and control, and other cooperative programs, without first determining what the needs were or the percentage of participation by the States.

By these policy statements and other methods we are now attempting to define the area of responsibility, and then attempting on both Federal and State levels to get the necessary funds to carry out our respective responsibilities.

We must understand, however, that the problem of having sufficient funds to meet all requests does not differ much on the State or Federal level. Apparently some State officials do not realize this but just think the Federal Government has an unlimited amount of money. This was brought out quite clearly recently in the brucellosis program.

You perhaps have heard that because of shortage of funds several States have reduced their appropriations, including funds for brucellosis. Several State Departments of Agriculture have informed us of this situation and have asked for a larger allocation of Federal funds to offset their States' reductions. The Federal Government is in the same boat--it cannot appropriate more money without either increasing taxes or increasing the national debt.

If we are to meet the demands for new programs, we either must have more funds or must discontinue some of the things we are now doing.

For several years I had been for giving more authority to States. However, a committee of the National Association of State Departments of Agriculture of which I was chairman reported several years ago that responsibility for cooperative programs should rest with the agency in line with its financial contribution.

Some States ask for more Federal funds but want less Federal dictation. There is, however, a definite need for the Federal Government being in the picture. I show you here a map. You will notice there are points in 16 States closer to Cherokee County, N. C., than to Dare County. Insects and disease are not respecters of State lines.

It is interesting to note what can be done in a State if they put enough effort into it. North Carolina was the first State to be certified as being modified free from brucellosis. Seventeen years later only two States within the area shown have been certified. I cannot see much reason why all 16 States could not have achieved the same position as North Carolina if the same State effort had been put into it. I believe I am safe in saying the benefits to North Carolina farmers have far exceeded their cost of the program.

Well, let us get back to tuberculosis. Here I can speak from experience, as I lost a herd to tuberculosis 35 years ago. I learned the hard way. After having five clean tests I added a cow without checking the health certificate, and in less than 2 years nearly the whole herd was wiped out.

More recently, a friend of mine added two cows that had not been tested for TB for 3 years, and in a short period of time he had 43 reactors. I am wondering if we have become complacent or if other problems have developed.

A short time ago a delegation of farmers who had several reactors called at my home, questioning the accuracy of the TB test. They seemed amazed when I said I would rather take out a questionable reactor, even at the risk of her not having TB, than to leave her in the herd and take a risk of exposing other animals. After some discussion they agreed that this was a good practice to follow.

I want to call your attention to an article in the Rhode Island Agri-Con News by Dr. T. J. Grennan, Jr., which describes the position we are in. Dr. Grennan writes:

> "It was felt that when all states were classified as modified accredited, prior to 1940, the time had come to look at one another and to extend congratulations on the tremendous progress that had been made in eradicating this disease.
>
> "At this time, a new 'disease complex' appeared, and rapidly spread through the livestock industry and its allied branches. This disease complex is often referred to as complacency....
>
> "The majority of those graduated from our Veterinary Colleges, within recent years, have entered various fields of veterinary medicine with the feeling that bovine Tuberculosis was practically a disease of the past, and did not present a problem today. Very little emphasis was placed on this disease in the Veterinary Colleges.
>
> "Some persons have publicly charged that continued Tuberculosis testing of dairy cattle is wasteful, and in view of the so-called 'practical elimination of the disease', is unwarranted. Many of the critics have felt that Tuberculosis testing should be done following the discovery in the slaughter houses, and tracing these active cases back to the herd of origin. The situation as it exists today in some areas points out the fallacy of this type of a disease control program. The critics to continuing an all-out eradication campaign have been answered.
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> "All cattle in Rhode Island are tested each year for Tuberculosis. This program, and the strict import regulations, should serve to prevent any dramatic increase in this disease among our cattle herds. Many of the larger states, with a heavy cattle population cannot financially afford annual testing. Their present situation shows, however, that they cannot afford to restrict expenditures in disease control unless they are willing to sacrifice the tremendous effort and expense of the past 20 years."

Just recently I visited the Island of Guernsey. They claim to be entirely free from tuberculosis and brucellosis. They credit their success to the fact that no cattle are permitted to enter the Island. This is a case of complete isolation, which I think we can agree is very important in the control of disease

In the United States we cannot have complete isolation of States, and it is difficult to have isolation of herds. But we can be more strict in the adding or intermingling of cattle.

It will take State-Federal teamwork to clean up this increase in TB infectio that has developed in some parts of the country.

STATUS OF TUBERCULOSIS ERADICATION IN THE UNITED STATES

A. F. Ranney, Chief Staff Officer, Tuberculosis Eradication,
Animal Disease Eradication Division, ARS, USDA, Washington, D. C.

We have met here this week to face a real task. There is no reason to minimize the job ahead of us or, on the other hand, to overemphasize the problems that must be overcome as we approach our goal of tuberculosis eradication.

It is easy for us to say that tuberculosis has not been eradicated because someone somewhere is not doing a good job or that some phase of the program is failing.

It is also easy to say that "the percentage of reactors in my State is lower than the national average" or that "we don't need to be concerned, because our counties are up to date on accreditation."

There are any number of offhand statements that may tend to get us away from the simple fact that tuberculosis has not been eradicated, and that is our job.

We can go back into the literature and find references to most of the major problems that are confronting us today. We make many suggestions in an effort to temporarily get around a specific problem, and then we may find that the suggestion or trial procedure does not prove as sound in the long run as we hoped or anticipated.

It is my privilege to review with you the status of the tuberculosis eradication project today:

During the fiscal year 1959 just past, 8,187,161 cattle were tested to find 18,914 reactors. This is the smallest number of cattle reported tested since 1945. But the percentage of reactors found in 1959 was 23, the highest since 1946.

The percentage of reactors slaughtered in 1959 that failed to show lesions of tuberculosis, 76.6, equaled the figure for fiscal year 1958.

It is interesting to observe that the number of reactors found in 1959 was greater than in the previous year by approximately 3,500 with an increase of approximately 800 lesion cases among the reactors slaughtered.

If we deduct the number of no-gross-lesion cases found (2,838) in herds containing lesion reactors and those reported with skin lesions (694), the percentage of no-gross-lesion reacting animals not known to have associated with lesion reactors would stand at 57.6 percent, or 19 percent less than the 76.6 percent no-gross-lesion cases.

There is no need to remind this group that the no-gross-lesion reactor has continued to be a sensitive subject and one that must have a lot of

careful study. We have reason to be cheered by the fact that scientific studies are presently being directed toward a solution.

It is interesting to observe that the percentage of no-gross-lesion cases varies widely among States. Even in those States considered to have major tuberculosis problems, the rate of no-gross-lesions may vary from year to year as much as 41 percent -- from a low of 44 percent to 85 percent, or higher.

Confidence in our modified accredited areas is generally lacking. Only eight States (California, Idaho, Louisiana, Nebraska, New Mexico, Tennessee, Utah, and Washington) will accept, without restriction, other than by a certificate, cattle that originate from such areas.

The livestock industry has reason to expect that the modified accredited area status will eventually be given more general recognition in accepting, without restriction, cattle from all such areas that are moved interstate accompanied by official certificates.

It is obvious that the provisions of the accepted program must be fully complied with in all States. Several counties in a few States are overdue for reaccreditation. It is imperative that delinquent counties be brought up to date as soon as possible in accordance with accepted standards. Otherwise, approved procedures fail to merit confidence and respect. Progress is being made in reducing the number of overdue counties. In November 1957 there were 347 overdue counties as compared to 136 on July 1, 1959.

Several States have taken very definite steps to work out their county testing program on a continuing basis, to facilitate a 100 percent coverage of herds. At least four States (Indiana, Iowa, Ohio, and Vermont) have scheduled testing in designated townships each year so that at the end of the reaccrediting period all herds will have been tested.

This results in a more uniform annual workload for accredited veterinarians and, in cases where counties are responsible for financially supporting the program, is equally helpful toward uniform annual fund requirements.

In 19 States a considerable portion or all of the retesting of infected or suspect herds is assigned to accredited veterinarians. In some of these States this may be in line with the program requirements in view of the number and location of full-time State or Federal veterinarians assigned to the project. It would appear, however, that the testing of these herds by regularly employed veterinarians would, whenever practicable, be beneficial to the program.

Only a few years ago, limited attention was given to tracing to the herds of origin animals that reacted to the tuberculin test or showed lesions on regular kill. It is most gratifying to observe the attention and increased interest that are now being given to this work. Ways and

means are being worked out that should result in even greater efficiency in locating foci of infection by tracing diseased animals and following up on those exposed.

It is quite apparent that regular veterinary personnel are regaining interest and enthusiasm for tuberculosis eradication. State and Federal livestock sanitary officials generally are developing a deeper interest and greater sense of responsibility for the project. This is most encouraging and must be maintained until the last tuberculous animal is slaughtered.

More attention is being directed toward keeping all veterinarians cognizant of their responsibility to the livestock industry in eradicating tuberculosis. This is resulting in better testing techniques and reporting procedures.

Most of the veterinary schools with the assistance of local State and Federal officials are holding demonstrations of tuberculin reactions in artificially sensitized cattle. This has made it possible for senior students to witness the injection of tuberculin into these animals by experienced operators and to observe and palpate the reactions that have resulted. At several schools students have an opportunity to inject animals as part of their clinical training.

It is encouraging to note that these demonstrations have been extended to meetings of veterinary associations and veterinary short courses, where they have been well received by the profession.

The Department of Agriculture's Meat Inspection Division has prepared an excellent training guide for those making post-mortem examinations of cattle that have reacted to the tuberculin test. This organized guide is being used by Federal, State, and municipal meat inspectors. Selected field veterinarians are being trained, at designated establishments in reactor post-mortem techniques. These employees will work with local inspection agencies in developing uniform inspection procedures for all tuberculosis reactors.

To close out the remaining reservoirs of infection, it becomes increasingly necessary to bring all phases of the program closer to perfection. Surveys are under way or being planned in an attempt to determine the incidence of tuberculin sensitivity caused by various types of tubercle bacilli. We must overcome factors that retard progress in tuberculosis eradication such as presence of paratuberculosis and exposure to the avian and human types of tuberculosis that are responsible for some of the no-gross-lesion reactors.

We must continue to carry on with the tried and true techniques and procedures that have stood the test of time -- while we work diligently for new procedures that may be developed, proven, and perfected to meet the changed conditions of the day.

HISTORY OF TUBERCULIN AND TUBERCULIN TESTS AND THEIR RELATIONSHIP TO THE DIAGNOSIS OF TUBERCULOSIS

Howard W. Johnson, Director, Animal Disease and Parasite Research Division, ARS, USDA, Beltsville, Md.

Tuberculin is one of the best diagnostic tools yet prepared by man! Before the large-scale production of tuberculin with synthetic media (synthetic tuberculin) by the USDA (1933), the testing of cattle and other animals was carried out with tuberculin made essentially in the same manner as the original product, produced by Robert Koch, now more than 65 years ago. It is true that, during the years that have elapsed since Koch's original announcement, many different forms of tuberculin have been proposed for use in both veterinary and human medicine. Many of these products were intended for use in the treatment of cases of human tuberculosis. Koch himself proposed such preparations, which were made by grinding and extracting components of *Mycobacterium*. Tuberculin prepared by growing *Mycobacterium* on synthetic culture media was used in human medicine and, on occasion, in veterinary medicine, for treatment. *However*, no form or modification of Koch's Old Tuberculin (O.T.) has been used in such large quantities and with such outstanding results as that made in synthetic media by the USDA.

There was no question of the remarkable efficiency of the Old Tuberculin of Koch. It enabled us to reduce bovine tuberculosis in the United States between 1917 and 1932 to a fraction of its early prevalence. In 13 States, the disease was decreased to a point where it ceased to exist as a menace to the dairy industry and to the public health. However, it must be stated that this, like other biological tests, is not perfect. There is a small percentage of instances where it has not been possible to find lesions of tuberculosis in reacting cattle, as well as where tuberculous animals have failed to react on a single test. Research has continued with the objectives of reducing this small percentage of *possible* error and of developing other tests.

Koch's Old Tuberculin.--In order that the differences between the synthetic tuberculin, which has been used exclusively in eradication programs since 1933, and the O. T. of Koch may be made quite clear, it seems desirable first to describe very briefly the Koch method of producing tuberculin.

A clear broth is prepared by extracting lean beef or veal with water. To this, 1 percent of peptone is added. The crude peptone is obtained by subjecting either meat or the casein of milk to partial peptic digestion so that it consists of a mixture of proteins. To the broth containing peptone, there are added 0.5 percent of sodium chloride and from 4 to 7 percent of glycerin. The mixture, which is generally referred to as glycerinated broth, is placed into flasks, sterilized, and inoculated with pure cultures of the *Mycobacterium tuberculosis*. In some laboratories the bovine type is used, but in the United States the human type is used. The bacteria grow on the surface of the broth, forming a film or pellicle which gradually covers the entire surface in about 6 to 8 weeks. The *M. tuberculosis*

grows actively on such a broth for only a few weeks. At the end of approximately 2 months, broth cultures of the _Mycobacterium_ are sterilized and the dead bacilli are removed by filtration.

A clear, sterile filtrate, concentrated to a desired strength and containing a suitable preservative, constitutes the tuberculin that was used for testing cattle in the United States. All of the O. T. produced by this method in the USDA was concentrated in such a manner that the final product contained 40 percent of the original volume of the culture fluid. All the O. T. produced and used by the USDA for the intradermic test contained 10 cc. of O. T. in each 40 cc. of the final product, or 25 percent by volume. It is, therefore, evident that the final product used for testing cattle contained not only the suitable substances derived from the growth of the tuberculosis organism on broth but also any portions of the culture medium which had not been used up during the growth of the _Mycobacterium_.

It was generally understood that the composition of the Old Tuberculin was extremely complex. It always contained considerable quantities of unused glycerin. In addition, there were present unused nitrogenous substances derived from the beef, as well as similar nitrogenous protein materials derived from the peptone which is added to the broth.

The synthetic medium.--Chemists and veterinarians working for the USDA conducted detailed studies for many years in an effort to devise an entirely synthetic medium for the growth of the _M. tuberculosis_. It was in the early 1930's that this synthetic medium was made possible by the continuing research of the Department. The synthetic medium constituents are asparagin, dipotassium phosphate, sodium citrate, magnesium sulfate, ferric citrate, dextrose, and glycerin. Since the early thirties, this simple synthetic medium has been used internationally for growing _Mycobacterium_, the product of which has become known as synthetic tuberculin. This tuberculin has been more uniform and more specific than Old Tuberculin.

The following three facts are very worthy of note:

1. The growth of _Mycobacterium_ is three or four times more abundant on the synthetic than on the old broth medium;

2. The proteins of the synthetic culture filtrates are derived entirely from the _Mycobacterium_; and

3. The constituents in the synthetic medium are entirely used except for a small amount of mineral salts and a trace of glycerin.

Many investigators have claimed, and it has been conclusively shown by the fruitful researches of Long and Seibert, that the active substance in synthetic tuberculin is a protein derived from the _M. tuberculosis_. Since the cultures on the synthetic medium contained no protein except that derived from the _Mycobacterium_ and since the growth of the _Mycobacterium_ was very luxuriant, it seemed probable that tuberculin prepared from such

cultures might be not only more potent but also more selective and less likely to result in nonspecific reactions than the O. T. of Koch. Many tests have been conducted over a considerable period of years in standardizing synthetic tuberculin and comparing synthetic tuberculin with O. T. It has not been possible to detect any significant differences in the reactions of cattle obtained with synthetic tuberculin of full strength and one diluted 25 percent, but differences are detectable with one diluted 50 percent. This may seem to be a rather crude significance test; however, this degree of accuracy has proved to be biologically sound. For details of comparative test on cattle with synthetic tuberculin and O. T., reference is made to Dr. Dorset's paper published in the Proceedings of the 37th Annual Meetings of the United States Live Stock Sanitary Association in December 1933.

The tuberculin reaction is generally accepted as a phenomenon of delayed hypersensitivity (allergy) due to the sensitization of the body tissues to the products of growth of the mycobacteria. The reaction may be either systemic or local, depending on the way in which tuberculin is applied to the tissues of responsive animals. When tuberculin is injected subcutaneously, it produces a local lesion at the site of injection and a systemic reaction which consists of fever and general symptoms of intoxication, and occasionally death. Experience has established the highly specific nature of the tuberculin reaction. It is recognized, of course, that every case of tuberculosis, either in man or in lower animals, will not necessarily give a positive reaction to tuberculin and, conversely, positive reactions are occasionally observed in apparently healthy persons and cattle. Because of the inadequacies of macroscopic and microscopic methods of detecting the presence of tuberculosis, we tend to infer inefficiencies of the tuberculin tests.

The subcutaneous test was the one most extensively used in the country and in certain continental countries, but it has been almost entirely superseded by the intradermal test for official purposes in those countries in which a serious attempt has been made to control tuberculosis in cattle. The ophthalmic, or conjunctival, tuberculin test of Wolff-Eisner, Calmette, and Guerin has been discarded for use in man owing to the severity of the reactions which it sometimes produces; however, the test is still employed in cattle to a limited extent. In practice, the test has not proved significantly reliable to warrant its use alone. Nevertheless, it is used occasionally in conjunction with some other method.

During the past three or four decades, considerable use has been made of and value shown in the intradermal test for detection of tuberculous infection. It was shown by Von Behring in 1899 that the skin of a tuberculous bovine was responsive to tuberculin, and since that time various methods of applying the intradermal test have been employed. The value of the intradermal test in providing clear-cut evidence of allergy to tuberculosis was plainly demonstrated by Moussu and Mantoux (1908), who described the type of response which might be expected in tuberculous and nontuberculous animals. As the reactions to the intradermal test can be read with a considerable degree of accuracy, the method has been in

great favor for 30 years or more, and has practically replaced the subcutaneous test.

The techniques of Christiansen and Stubb (1910) consisted of introducing concentrated tuberculin into the deeper layers of the dermis. An area on the side of the neck was prepared, and a preliminary dose of 0.1 cc. was injected into a fold of the skin. At the 48th hour, a positive response in the form of a soft edematous swelling resulted, but if the reaction was of a nature of a hard circumscribed swelling, which characterizes a negative reaction, a second dose of 0.1 cc. was introduced into the center of the primary swelling. Investigations by Duxton and McNaulty (1928) seemed to indicate that this procedure would considerably assist in the detection of tuberculous infections in all its forms. This did not prove to be the case; consequently, the procedure has been discarded.

The Stormont test was developed in Northern Ireland by Kerr, Lamont, and McGirr. It involves two intradermic injections in the same site 7 days apart. These injections were made in the cervical area. The second injection was observed in 24 hours, and an increase in skin thickness of at least 5 mm. more than that caused by the first injection constituted a specific reaction.

The intradermic caudal fold test, intradermic vulva test, and the intradermic cervical test are all further variations in the use of intradermal testing procedure. All of the test procedures will be demonstrated to this group late in the week.

The ophthalmic test is still another test which has over the years received considerable attention. It is usually conducted as follows: A sensitizing dose of tuberculin or a disk is installed underneath the eyelid of the animal. After an interval of 72 hours, a second dose of tuberculin or a disk is installed in the same eye. The disks usually contain twice the regular intradermic strength of tuberculin. Four readings are made at 2-hour intervals after the installation of the second disk. A positive reaction is indicated by copious lacrimal secretion, usually mucopurulent in an extreme reaction. It is reasonable to suppose that the hypersensitivity which develops in the tissues as a result of the invasion of M. tuberculosis manifests itself in a similar manner in all infected subjects, but it should be recognized that there are slight differences in the allergic response of different species.

While there is no reason to believe that there are any significant qualitative differences in the results of the intradermal test in the various infected species, there are quantitative differences of skin sensitivity. Attention may be drawn to certain peculiarities of the intracutaneous tuberculin test in cattle since some interesting differences have been observed in the bovine subject as compared with man. Comparatively large doses are required to detect all infected cattle, some of which are only slightly sensitized. This is in marked contrast to the appreciably smaller amounts needed to reveal tuberculous allergy in man. Although this difference may be dependent in part upon

the relative thickness of bovine skin, which ranges from 3 to 12 or 15 mm., it is unlikely that this factor is entirely responsible for the relatively lower degree of sensitivity in cattle. Another possibility is that the cutaneous tissues in the bovine subject are less responsive than those in man. Cutaneous reactions in man are frequently obtainable with tuberculin diluted 1:1,000 or more. Similar responses can be observed in guinea pigs at the appropriate stage of tuberculosis, whereas in cattle it is usually necessary to employ concentrated tuberculin. Although hypersensitivity does not occur frequently in tuberculous cattle, positive reactions of 40 to 50 mm. may be induced in them at the time of maximum sensitivity with dilutions of 1:100, or even 1:1,000, of regular tuberculin, whereas in other cattle with less sensitivity, 0.1 cc. of undiluted tuberculin is required to produce a reaction of equal size. Consequently, the response to concentrated tuberculin in a large proportion of the reactors in an affected herd is of a moderate intensity.

I will briefly discuss some of the research on tuberculosis that is presently under way and explain the reasons for these lines of approach. Research will, of course, increase our knowledge of tuberculosis. However, I would like to point out that we should not pin all our hopes on techniques not yet developed. Let's first be certain that we are using all methods and experience available to us to the best possible advantage. As an example--the intradermic injection of tuberculin must be performed carefully and accurately. In applying the test we are dealing with a degree of allergy in the animal that demands extremely critical and meticulous techniques to detect. Unless the test is carefully applied and the results interpreted with extreme care and professional judgment, it is of little value. Future research will not compensate for careless application and interpretation of the present tuberculin test.

The tuberculin presently in use is the best available. It is standardized so that the recommended dose will produce a reaction in most infected animals. Occasionally, infected animals may fail to react because their sensitivity to tuberculin is so low that the standard dose will not detect it.

In research on tuberculosis we must remember that in addition to Mycobacterium tuberculosis var. bovis there are other bacteria belonging to the genus Mycobacterium. This genus is a manmade classification of a number of species of bacteria that have certain characteristics in common and the species "tuberculosis" is further subdivided into three varieties. These closely related bacteria contain certain proteins which appear to be identical. For instance, a tuberculin made from M. tuberculosis var. bovis contains certain protein components which might elicit reactions in animals infected with other species of Mycobacterium. However, we think that each species and variety also contains proteins which are found only in that species or variety. Such proteins would be called specific proteins. If these specific proteins could be isolated chemically we would have a specific diagnostic product for cattle infected with bovine tuberculosis. We are working on the hypothesis that such proteins are present and can be isolated.

We have been discussing the bacteria and their products, now let us discuss the infected animal or host. Since it is infected with a species or variety of Mycobacterium it may be sensitized by the various proteins composing the bacillus. This may complicate the problem of developing a specific test product. Research in this country and Great Britain has shown that certain protein fractions of tuberculin when injected into artificially sensitized animals are quite specific. However, when these same products are used in the field on naturally infected animals the specificity is not better than the present tuberculin. One of these products is a purified protein derivative of tuberculin (PPD).

If a pure protein that is specific for bovine tuberculosis can be isolated we may have one of the answers to the problem of tuberculosis eradication.

Another line of research currently in progress is the hemagglutination test for tuberculosis. Dr. Larsen and his associates at the ADP Regional Animal Disease Laboratory at Auburn, Ala., have been working with this test for several years. The present status of the work suggests that we are approaching a practical test which may do for tuberculosis what the ring test has done for brucellosis. If the hemagglutination test can be perfected, blood samples obtained for routine brucellosis testing can be checked by this particular test for the purpose of spotting tuberculosis infection and following with intradermic tuberculin testing of the suspected herd.

The hemagglutination test is conducted as follows: Red blood cells of sheep are sensitized with PPD tuberculin or a similar product. Serum to be tested is first exposed to nonsensitized erythrocytes to remove certain nonspecific material. The sensitized cells are then exposed to the serum and incubated to complete the test. Sera from infected animals agglutinate the sensitized cells.

Another promising bit of research has to do with tissue culture. HeLa cells are now being used to grow Mycobacterium tuberculosis var. hominus. This method appears to have advantages over those currently in general use for isolation of this bacillus. Additional work is now under way on this procedure which may be helpful in the overall problem.

In closing, I should like to emphasize that more knowledge of tuberculosis is needed to assist us in eradicating the disease. But, in the meantime, it is necessary to make full use of all knowledge presently available. An example of this is the Cache Valley problem in Utah. Originally it was thought that tuberculosis did not exist in that locality and tuberculin reactions in cattle there were from unknown causes. However, a careful study of the problem revealed the presence of both avian tuberculosis and Johne's disease, both of which may cause animals to elicit reactions to standard tuberculin.

A POSITIVE APPROACH TO TUBERCULOSIS ERADICATION

G. S. McIntyre, Director,
Michigan Department of Agriculture, Lansing

In the fall of 1954 the Michigan Department of Agriculture started a county wide test for tuberculosis in cattle in Sanilac County. It was soon evident that there was an increase in the incidence of the disease above that which was expected or which had been found on tests in other counties. The number of reactors was running at better than 2 percent in Sanilac County, and when testing was completed an average of 1.8 percent of the cattle showed up as reactors. Shortly thereafter another county was opened up. The number of reactors in one township alone was better than 5 percent, and in certain areas of that township the infection rate was as high as 8 percent.

In October 1958, a certain herd of cattle was tested. Four children from that farm family were tested for tuberculosis in their school on the same day. Thirty-seven reactor cattle were found. The four children also reacted to the tuberculin test. In January 1959, another herd was found with 22 reactors. The tenant was positive to tuberculosis when X-rayed. In April 1959, a reactor was found on another farm, and the herd owner's son was positive to a tuberculin skin test.

How many times has this happened? How many times can this happen? No one knows the answer. The fact remains, though, that it does happen. This places a human factor on the tuberculosis eradication program, and adds a note of urgency to getting the job done.

Since the inception of the tuberculosis testing program the State and Federal governments have expended approximately $350 million on testing for and eradication of tuberculosis. One would think that after these many years and that much money tuberculosis would have been eradicated. The fact remains that tuberculosis has <u>not</u> been eradicated. And the very unfortunate fact exists that tuberculosis is actually on the increase.

I am glad that this conference has been called. It will not only bring attention of the tuberculosis problem to the many people and to all the States, but it will also bring to each the latest information available and will develop a uniformity in the techniques and methods used. This is extremely important if we are to have a uniform testing and eradication program over the entire United States, and a program which all of us will have confidence in and which will result in our desired goal of eradication.

It is not my purpose here to discuss the technical aspects of tuberculosis eradication. But I believe there are some basic principles involved as to why we still have bovine tuberculosis in the United States. I do not, however, want to leave the impression that we are <u>not</u> proud of past achievements.

This program was started in 1917 and after approximately 20 years the entire country was declared a modified accredited tuberculosis-free area. Compare this condition with similar conditions in European countries. In Germany the infection rate is so high that the Army ships its personnel milk requirements into Germany from the low countries. A similar situation exists in France.

Accreditation of the United States has saved livestock people many millions of dollars. Without a doubt it has saved thousands of people from becoming diseased through the use of milk from infected animals or association with them. This is a major achievement, and credit for it belongs to the veterinary profession, the livestock people, the livestock sanitary officials in each of the States and other Federal and State officials.

But self-glorification can be foolhardy. This was brought shockingly to the attention of Michigan people 4 years ago. It was again brought shockingly to our attention when we noticed that 20 to 30 percent of the total reactors condemned in the United States each month come from the State of Michigan. In May of 1959, out of 2,066 reactors located in the United States, 1,026 came from Michigan. Why was this? In the first place, I do not believe that bovine tuberculosis in Michigan is comparatively that bad, but Michigan has materially increased the number of cattle being tested and has been discriminating in its appraisal of the reactions to the test. We believe this has raised the percentage of the total reactors that have come from Michigan.

But why do we have this increase in the percentage of reactors over previous years? Why did the percent of cattle that react to tuberculosis increase from 0.3 percent to 0.9 percent during the last 5 years? Why is it that we have found one reactor animal every 2 hours during the last year? A critical analysis of the past can quickly tell us why this is true.

Michigan was accredited in 1932, and almost immediately the importance and the seriousness of tuberculosis was forgotten by the livestock men, by the veterinarians, by State and Federal control officials, and the educational people. Bovine tuberculosis was considered a thing of the past. It was a rude awakening when we found that the disease all of us thought to be unimportant now was one of major proportions. Why did it happen? We in Michigan believe that this situation is due to many reasons and not to just one:

1. Lack of Proper Educational Program

After the accreditation of the State, the educational program was dropped except for that kind of a false program which led people to believe that there was no need to worry further about bovine tuberculosis. I believe this has been true in almost all of the States. However, a new generation is continually taking over, and the new generation that came up after the problem of tuberculosis was practically solved in the 1920's and 1930's knew little about the seriousness of the problem. They had

not experienced it. The lack of an educational program resulted in lethargy and carelessness on everyone's part.

2. Lack of Aggressive Attitude on the Part of Veterinarians, Farmers, and Federal and State Control Officials

Because of the lack of an educational program veterinarians, farmers, and Federal and State control officials did not realize the seriousness of decreasing the number of cattle tested. Tuberculosis was considered a thing of the past. Appropriations made were not always used, even though the number of cattle that should have been tested were not tested. Reaccreditation of counties fell behind schedule.

3. Lack of Training

Veterinarians did not always test all of the animals in a herd. They failed to test animals which were out on pasture. They skipped farms on the area test. Some of our highly infected herds had not been tested for a period of 10, 12, or 14 years, even though the counties in which the herds were located had been tested once or twice during those periods. We found tests were not always read, because the veterinarians thought there was no use in reading them--there would be no reactors anyway! We found a lack of uniformity of the interpretations placed on the reactions to the test.

4. Lack of Proper Import Regulations

Requirements on importation of cattle into the United States were not as strict as they should have been. This, however, was changed several years ago and I do not believe that we are importing infected animals into the country at this time. It was, however, I am sure, a factor in the increase in the incidence of tuberculosis in Michigan.

5. Lack of Sufficient Numbers of Cattle Tested

There were not enough cattle tested yearly. I think this complaint could well be carried into many other States. In the beginning of the program we in Michigan tested all cattle in a county each 3 years in order to meet accreditation requirements. This was later reduced to one-half of the cattle in a county each 3 years so that all of the cattle would be tested each 6 years. Added to this, however, was the trace back from infected animals found in slaughterhouses. I am told in some places counties are reaccredited based on a test of 10 percent of the herds every 3 years, and sometimes it is the same 10 percent of the herds that were previously tested.

6. Improper Reaccreditation of Counties

Counties are reaccredited each 3 years or each 6 years if certain conditions are met. These conditions had not always been fully met in many Michigan counties, and I feel they may not have been met in many

counties outside of Michigan. Yet those counties have continued to enjoy accredited status, and the cattle in those counties have moved under the privileges afforded by an accredited-area status. I am sure that the lack of compliance with reaccreditation rules has had a significant influence on the increase of bovine tuberculosis.

7. <u>Lack of Sufficient Control of Cattle Movement</u>

There was an accelerated movement of cattle following World War II. Most diseases are spread following the movement of infected animals to associate with animals that are not infected.

If we are ever to eradicate tuberculosis from the United States, we must develop a sound eradication program and follow it. This is not to say that the program which has been followed in the past has not been sound, because the achievements of the kind made under this program could not have been made under circumstances other than a sound program.

However, during the past 5 years in which Michigan has had an accelerated program, there have been several conditions noted which disclose that a reappraisal of the entire tuberculosis program must be made. There must be a reassessment of it. At one time, 75 percent of all the bovine tuberculosis was found in the nine central Great Lakes States. Today it is found in increasing numbers in both the Southern and Southwestern States. This is probably due to the movement of cattle from the Northern States to those Southern and Southwestern States.

There are changing patterns in the disease. Fewer lesion cases are being found today than in the past. Why is this true? Is the disease less virulent than it used to be? Is there a change in the type of organism that we call the tuberculosis organism? Is what we are now calling tuberculosis caused by other types of organisms or some other unknown factors? These are disturbing things which have made their appearance within the last few years. These are things which must be answered. These are problems which I am glad are now being recognized, and will be answered through proper research. Toward this end the United States Department of Agriculture has set up research projects in cooperation with both Wisconsin and Michigan. From research will be given the answers to these questions.

In spite of the many problems, we in Michigan have not become discouraged over eradication of tuberculosis. Our experience indicates that it can be done under present known methods. But we are sure that research is going to point a way to new, easier, and cheaper methods of doing it. There are, however, certain principles involved in this or in any other program which must be considered and adhered to. These are:

1. <u>A Determination to Eradicate Bovine Tuberculosis</u>

In my opinion the only proper attitude one should have toward the tuberculosis program is <u>complete eradication</u>. Properly applied, the tests and methods of the past have shown that the disease can be held at very

low rates of infection. With knowledge and techniques that will be available in the future, tuberculosis can be eradicated. Any lesser goal is unacceptable.

2. State Responsibility

It is my opinion that any disease control program is the responsibility of the State. The State must take the responsibility for developing and executing a program. I believe it is the responsibility of the Federal Government to help the States in their problems and to provide leadership in developing effective programs, and to protect the States from the importation of infection from outside of the country or from other States. There must be the closest kind of cooperation between State and Federal personnel. For a program to be effective there must be no jealousies, there must be no rivalries, there must be complete cooperation. A farmer cares little whether a veterinarian is employed by the State or Federal Government. He wants the disease eradicated. It is he who suffers if there is not complete cooperation between the agencies.

3. Administrative Organization

There must be a proper administrative organization developed. One of the very first things we did in Michigan, after we discovered our incidence of tuberculosis, was to set up an administrative organization in which both Federal and State employees are thoroughly integrated. In some cases State employees are supervised by Federal men, and in other cases Federal employees are supervised by State men. There are no misunderstandings of supervisory authority. I am sure this has happened to some degree in many other states. Close cooperation between the agencies is essential.

4. Central Control

There must be central control of the entire program. One of the most useful things found in the tuberculosis eradication program in Michigan has been the IBM system of record keeping. Through this system the program can be directed from the State office. Prior to the inception of the IBM system the program was directed largely at the district level. This is not a satisfactory way. It was the decentralized record system which permitted so many herds to be missed on previous tests. Under the present control plan every herd must be tested or otherwise accounted for.

5. Personnel Supervision

There must be close supervision of all personnel. Both salaried and contractual veterinarians must be closely supervised. The tuberculosis eradication program will be only as successful as the supervision given.

6. Reaccreditation Standards

There must be a reassessment of the reaccreditation rules and adherence to them. The rules for reaccreditation must be reviewed and revised. There must then be strict adherence to the rules after they have been established. I am sorry to report to you that no longer will Michigan recognize the accredited status of counties as a basis for the movement of cattle without test. This is true whether those cattle moved intra- or interstate. Under a law just passed by the Michigan legislature, cattle must be tested for tuberculosis before they can move either within or into Michigan. They will have to be tested within 90 days for an individual animal, or they will have to come from an accredited herd or a herd of which all members have been tested within the past year and found negative. This requirement was demanded by the livestock people of Michigan and was passed by the legislature.

7. Training

There is a need for better training of the veterinarians in techniques of testing for tuberculosis. Too many veterinarians have graduated from Schools of Veterinary Medicine without having been fully trained in these techniques. This applies to both the full-time salaried veterinarian and the practicing veterinarian.

8. Animal and Human Health Liaison

There must be a close liaison between the Health Departments and the Departments of Agriculture. Whenever a diseased herd is found in Michigan it is reported to the State Health Department, which in turn refers it to the local Health Department. The local Health Department can then work with the families to determine whether any member of the family has contracted tuberculosis. Conversely, when the Health Department finds a farm family with tuberculosis, it is reported to the State Department of Agriculture so that the herd, if there is one on that farm, can be tested for the disease. This close liaison can be helpful in resolving problem cases of tuberculosis in human medicine as well as in veterinary medicine.

9. Quarantine

Infected herds must be quarantined, and these quarantines must be enforced. The infected herd must be retested at given intervals until evidence of infection no longer exists.

10. Education

There must be an extensive educational program. A new generation of livestock men has taken over the farm operations since the tuberculosis eradication programs were first initiated. This generation of livestock men do not fully understand and appreciate the dangers connected with bovine tuberculosis. This is a job for the Extension Service and a job

which should be undertaken by them in the immediate future. It is just as important that an educational program be conducted on tuberculosis at this stage as it is that one be continued on brucellosis. We know that when people understand the problems they will make efforts to solve them.

These principles, in my opinion, are necessary for a sound approach to an effective tuberculosis eradication program. There may be others. I am told that the tuberculin test is one of the most accurate diagnostic tests devised. We know its use has been effective in the past. Yet, after 40 years and an expenditure of $350 million, we are again concerned over the incidence of the disease. I have related why I believe this to be true. We must again embark on a positive program of tuberculosis eradication. In this day and age we can justify nothing less.

Last year's Tuberculosis Conference in Michigan started us on our way. This conference should do much toward helping all of us better understand the disease and the problems connected with it. It should help us develop a better and a more uniform program. I believe it is the responsibility of all of us here to reevaluate our programs, to reevaluate our techniques and our attitudes, and to decide that it is time to eradicate tuberculosis and not to live with it.

II. GETTING MAXIMUM RESULTS FROM TUBERCULIN TESTS

APPLICATION OF THE TUBERCULIN TEST

B. E. Knisely, Supv. Veterinary Livestock Inspector, Animal Disease
Eradication Division, ARS, USDA, Marion, Ohio

Gentlemen: I would like to spend a few moments discussing the word "application." We think of it as meaning to put something in, to put something on, or adding something to. After these, we seem to place a period, forgetting that they are put on, or put in, or added to for a purpose. If we had no purpose in mind when painting a house, for example, why not just stand back and throw the paint against the house? Of course, we wouldn't do that, because we would be putting it on the house for the purpose not only of improving the looks but also of preserving the wood.

Another meaning of application, and the one which I am going to use, is "The Practical Demonstration of a Principle": "Practical" meaning applying one's knowledge to a useful end; "demonstration" meaning displaying or exercising; "principle" meaning a law of nature or scientific rule. So, we come up with the meaning for application: "The ability to apply one's knowledge to a useful end by displaying or exercising a law of nature or scientific rule." In this case, the scientific rule is the tuberculin test.

There are a minimum of qualifications which each individual should possess. The first is a complete knowledge of the disease and some knowledge of the tuberculin test. There should be no excuse for not being

acquainted with this disease, as it has been known to man from almost the beginning of time, and there have been thousands of papers and pamphlets written about the disease, covering it from all angles. A knowledge of the disease serves twofold. It gives a feeling of confidence and places one in the position to talk intelligently with the people of the livestock industry. Many times I have heard people say when asked questions about tuberculosis, "Don't ask me, call the office," or "Let's call the district man." How can a person conduct a test or anything else of such vital importance if he doesn't possess a workable concept?

The next qualification must be arranged by the individual, within oneself. I do not know how it is done today; I do know how it was done 25 years ago, and it only took about 2 minutes to get it done. However, things have changed considerably in 25 years. A good job cannot be done unless one is in the proper frame of mind and has the will to do it and, once that is attained, proceeds to do it.

In our section of the country, we now conduct meetings before tuberculin testing is started. We attempt to iron out any discrepancies beforehand and have a practice session to see if everyone is properly briefed. Each person is given a pamphlet, and the detailed work contained therein is fully explained. We have found that one ounce of prevention is worth far more than several pounds of cure.

Preparation before going to the field to apply tuberculin tests is an absolute necessity. Every individual should acquire the necessary equipment and become acquainted with its use. The delicate 2-cc. tuberculin syringe should be carefully studied and handled to get the feel, then tightened before going to the field. Nothing is so embarrassing as for one to report to the field and suddenly discover the syringe is loose and leaking with no way to tighten it. Other equipment needed: Nose tongs or rope halter for restraint, alcohol, cotton, field pad, necessary forms, foot brush and bucket, disinfectant, and tuberculin.

One should have assistance in the form of helpers. Proper injections cannot be properly made unless there is assistance of some kind. A good helper is considered by most operators to be at least 50 percent of the job.

Before starting any tuberculosis work, industry itself should be solicited for its cooperation. Prior to TB testing, quite often men drive onto an owner's premises and say, "We are here to test your cattle, how about getting them in!" Now, if I were an owner I know what I would say, especially if I were busily engaged doing many other farm chores. There are several ways industry can be solicited or contacted--by mailing a card or letter, telephoning, or contacting directly. One thing I have found to "soften up" an area, county, or township, to renew interest and let them know what we are about to do, is to write several articles in the local newspaper from 4 to 6 weeks ahead of time. Then, possibly a week before the work actually starts, an article is put in the paper suggesting cooperation from everyone. This, some people in the profession might say, is humbling oneself. In my experience of some 25 years I have found this to pay big dividends.

I believe that each and every individual not well acquainted with the procedures and techniques involved in tuberculin testing should be assisted by someone well versed along these lines. I firmly believe that everyone who is desirous of doing tuberculin testing should have a minimum of 3 days of injecting and 3 days of observing under the guidance of an experienced operator. If longer time is needed to produce a good operator, then by all means it should be taken.

Proper restraint:--I didn't intend to talk about this subject right in this spot, but because of the time element perhaps I should, so we may look at the slides. It is my firm belief that no one can make a correct injection unless some type of restraint is used. Persons who say they can, should be challenged. These are the injections being "shot out into orbit," and we don't have any cows out there as yet. I know that an animal responds normally to the prick of a needle, and, Gentlemen, some of these big Holstein cows kick as hard as a mule. If we have the proper understanding of the disease, have the necessary qualifications, and have correct preparation, then by all means we should use some type of restraint in order to make the proper injections.

What a fine feeling of satisfaction and confidence one attains if he knows he has done as good a job as is possible. It creates good will and better association in the future, if the work has been done correctly and a clean bill of health is given an owner. He naturally gains a sense of confidence and satisfaction in what you have done. Even if reactors are found, that same confidence is attained if the work is done right. We guide many senior students today, and the initial or basic work we deem to be of vital importance. If we can get them to understand they must do a good job on injections, we needn't worry too much about the future work, as it then will follow in a normal and successful pattern.

Application of the Tuberculin Test of the Bovine Species

Examine the caudal fold area to determine any preinjection abnormalities. If so found, an inexperienced operator should make a notation of this to avert trouble in observation. Roll the caudal fold outward over the thumb or finger before injecting. This fold is the soft hairless skin on the underside of the tail head. Sufficient time should be taken to insure a scientific operation. Cotton used dry or dampened with alcohol should be used to clean the injection site. Stronger cleansing agents may cause irritation of the skin that could result in faulty observation.

The needle should be inserted between the layers of skin, and one should make certain that the insertion is far enough to prevent leakage after withdrawal. To prevent a subcutaneous injection, when inserting the needle lift the tip slightly until the needle is clearly outlined under the skin. The recommended dosage is 0.1 cc.

Vulvar injection--As a check test this site of injection is the lower half of the vulva. Again checking for abnormalities, the procedure is about the same as the caudal fold injection. The insertion of the needle

should be at the junction of the skin and mucosal membranes. One cc. is the amount of tuberculin used.

The needle is wiped off with a small pledget of cotton dampened with alcohol to insure the next injection free from contamination. The syringe is placed back in the carrying case and is ready for the next injection.

Tuberculin Testing of Swine

Tuberculin testing of swine is by the intradermic method. The soft skin near the base of the ear may be used, or the vulva injection as used in cattle.

Avian tuberculin may be injected on one side and mammalian tuberculin on the other side. Injections may be read in 48 hours and 72 hours.

It is not necessary to apply the test to all the animals in a herd, as it is more economical to send the whole herd to slaughter if infection is found. An exception is the value placed on purebred breeding stock, in which case the whole herd could be tested and the animals found infected removed.

All sheds, farrowing houses, lots, feeding platforms, troughs, and so forth, should be thoroughly cleaned and disinfected as for cattle and poultry.

Tuberculin Testing of Poultry

The application of a tuberculin test in poultry requires a little more patience than in cattle or swine.

Tuberculin testing in poultry is done by injecting into the skin of the wattle or the skin of the vent a small quantity of tuberculin, by means of a small syringe and needle. The amount of tuberculin injected is 0.05 cc. The swelling at the point of injection at the end of 48 hours indicates the presence of tuberculosis.

Only a few birds of an apparently healthy flock should be injected, as it is impractical to test all the birds because of the cost. However, in cases of purebred breeding flocks, the value of such fowls may justify the cost.

The Cervical Test

The cervical test will be used only in known infected herds or problem herds where the herd becomes difficult to clean up by use of the intradermal test. It should then be used only by prior approval from State-Federal cooperative agencies.

In its use 0.2 cc. tuberculin is injected intradermally. Animals so injected should be read at the 48- and the 72-hour interval. Highly sensitive animals may start showing a reaction at the end of 24 hours.

If the cervical test is deemed necessary, contact your department, get authority, and someone with experience along this line will be glad to assist you.

The cervical test should not be substituted for the caudal fold test on routine testing. We find this going on today because some folks don't care to get their hands soiled.

Gentlemen, I see my time has overrun, and I am indeed sorry we have to hurry through this, as there are may details which have to be left out; but perhaps some time in the future we will have the opportunity of being together again, and more time can be spent on these details.

Let's reaffirm our confidence in the tuberculin test, because when properly applied and interpreted it is one of the most dependable disease detection tests we have.

Finally, let's put forth the kind of effort in our tuberculosis eradication work that will gain the most with the least expenditure. Let's evaluate ourselves and our work, remedy the situation, if need be, and eradicate tuberculosis.

TRAINING VETERINARY STUDENTS FOR REGULATORY PROGRAMS

E. E. Leasure, Dean, School of Veterinary Medicine, Kansas State University of Agriculture and Applied Science, Manhattan

The title of my address, "Training Veterinary Students for Regulatory Programs," might suggest that the School of Veterinary Medicine, Kansas State University, makes a special effort in this respect. I do not wish to leave this impression however, because in reality the educational objective of the School is a very broad one. We are striving to give our students a good basic-fundamental education in all areas of veterinary medicine without neglecting any area. We believe that our student graduates generally are well informed and could step into any area or field of Veterinary Medicine.

We are not concerned with the production of specialists in the 6-year curriculum, because we are aware that additional education and training experience is a vital requisite in equipping a specialist.

Our undergraduates in veterinary medicine receive some instruction on State and Federal regulatory programs throughout the 4 professional years; however our greatest effort in this respect is put forth in the fourth year of instruction.

From the second semester of the second year onward, students in the courses of pathology, diseases of large and small animals, and

diseases of poultry are informed of the regulations relative to reporting infectious and contagious diseases and the importance of reporting these diseases to the proper Federal and State Officials.

In the fourth professional year, livestock and poultry disease regulations and regulatory programs are stressed in the courses entitled "Veterinary Ethics and Official Livestock Regulations," "Food Hygiene and Public Health I - II," and "Clinics III and IV."

The following represents a condensed syllabus of the instruction given in the first course mentioned:

I. Veterinary Ethics and Official Livestock Regulations.

State laws governing Veterinary Practice

Federal laws and regulations governing Veterinary Practice, such as Tuberculosis and Brucellosis testing and the issuing of health certificates.

State and Federal laws, rules and regulations relative to the movement of livestock and poultry. This section would include such items as: the person or offices charged with animal disease regulations; the importation and exportation of animals, poultry and animal products; the issuing of health certificates and charts; proceedings for condemned animals, appraisements and reimbursements; special regulations governing the shipment of diseased animals inter- and intrastate, sanitary and disinfection requirements of stockyards and vehicles used for transporting livestock.

Rules and regulations relating to viruses, serums, and analogous products.

Uniform methods, rules and regulations relative to Tuberculosis, Paratuberculosis and Brucellosis eradication and control.

Certain sections of the Kansas and the Federal Food, Drug and Cosmetic acts.

The Kansas livestock laws.

The Kansas brand laws and regulations.

Regulations and inspection requirements and community sales laws.

State and Federal laws, rules and regulations governing specific diseases, such as hog cholera, red water, Texas fever and pullorum disease.

Each year special lecturers appear before the class from the State Office of the Agricultural Research Service, the State Livestock

Commissioners Office and the Veterinary Section of the Kansas Department of Health. These lecturers discuss regulatory measures and enforcement, and the proper completion of health certificates, charts, and forms.

In addition to the above, once each year a veterinarian of the Agricultural Research Service, either of the Meat Inspection Division or Animal Disease Eradication Division, is brought to the Campus for an all-day session with the fourth-year class. Students are informed in advance to prepare questions for the individual's specific area, and the speaker is likewise informed that he may discuss any phase of his regulatory work. This has proved very beneficial.

II. Food Hygiene and Public Health Courses

These courses cover more specifically the State and Federal laws, rules, and regulations appertaining to sanitation and hygiene and all food inspection. Its condensed syllabus is as follows:

Meat Hygiene

Origin and source of meat
Antemortem and post-mortem inspection
Sanitation
Processing and preservation of meat
Federal regulations and their interpretation
Adulteration, labeling and misrepresentation of animal food products
Parasites transmissible to man from animals (meat) and their recognition
Diseases transmissible to man from animals or their products (Zoonoses)
Rodents and pest control in packing houses
Inspection exemption certificates
Food poisoning and food-borne infections
Poultry regulations and inspection
Fish and seafood inspection
Milk and dairy products inspection
Insecticidal poisoning of livestock
Fallout and radiation hazards to meat food animals

Public Health

Organization with emphasis on the veterinarians place in the service
Coordination of animal disease eradication with disease incidence in the human population--ex. Tuberculosis and Bangs Disease.
Three days are spent with meat and poultry inspectors in Kansas City Packing Plants.
One full day is spent in inspecting off-campus dairies and dairy processing plants.
Three afternoons of each week, fourth-year students under the supervision of a staff member, conduct antemortem and post-mortem inspection of all animals slaughtered in the University's modern slaughter plant here on the campus.

With the opening of the fall session this service will be expanded to include three additional afternoons at a local off-campus packing plant.

Off campus speakers are invited to speak to the class on poultry inspection, milk inspection and meat inspection.

The Assistant Chief (Dr. D. Decamp) of the Poultry Inspection Branch of the Poultry Division, AMS, has been a regular speaker for several years and has several films which he uses to illustrate his talks.

A pathological specimen display is prepared by the Kansas City Meat Inspection Station for the students to study, and the display represents 4 or more months' collection of material from the four largest plants in Kansas City (Armour, Swift, Cudahy and Wilson).

III. Courses in Clinics

The Kansas State University, School of Veterinary Medicine, is quite fortunate in having a rather large ambulatory clinical service.

Each day of the week, eight fourth-year students are assigned to abulatory clinic. The students, under the supervision of a staff member, in this way become acquainted with certain aspects of regulatory programs, such as tuberculosis and brucellosis testing, dairy inspection and the issuing of health certificates and charts.

Each Senior must be proficient in the application of the Brucellosis test.

Since 1950, the School has demonstrated to the Seniors the Tuberculosis testing of sensitized animals.

This in brief represents a summary of the training program given at this institution.

THE PRACTITIONER AND TUBERCULOSIS ERADICATION

Thomas P. Crispell, Sr., The Parsons Stock Yards
Parsons, Kans.

Fellow Veterinarians--Livestock Sanitarians and Listeners:

The writer, though being the only practicing veterinarian from Kansas on the program, is not speaking officially for his Kansas colleagues, but is giving you the viewpoints and opinions of a general practitioner who has made a study of tuberculin testing of bovines and one who considers this phase of veterinary practice most interesting and remunerative.

My first dealings with tuberculin testing of bovines was at Pine Plains, N. Y., in 1913 and 1914. At that time, I was a dairy hand in the Certified Milk Dairy of Briarcliff Farms. This herd was under the supervision of Dr. Knapp of Millbrook, N. Y. The test at that time was conducted thermally. I, as a dairy hand, introduced and withdrew the thermometers, with Dr. Knapp making the readings. These readings as you all know were made at 2-hour intervals. All of us soon acquired considerable lay knowledge about temperature curves and so on, especially as they applied to our favorites in the milking cows. Along with this we came to recognize the arched back, increased respiration, and scour that so frequently indicated a reaction without benefit of thermometer readings.

As a senior in the Chicago Veterinary College, Dr. Boyd came down from Minnesota and Dr. Jungernem from Kansas to give us intensive short courses in sterility with advice on tuberculin testing thrown in.

After graduation our testing was done both thermally and intradermally for a while, but soon all thermal testing was abandoned and we depended solely on the intradermal test introduced by Dr. Luckey of Missouri and the opthalmic test used as an aid when the intradermal reading was inconclusive or when we were dealing with a problem herd.

I assisted with the Statewide Kansas cleanup under the supervision of Dr. Henry M. Graefe and the Honorable J. H. Mercer. Special technique instruction was given me by Dr. Lash of the U.S. Bureau of Animal Industry. My early tuberculin testing was supervised and I was thoroughly coached by Drs. E. C. Cannon and F. B. Jones of the Bureau and Dr. John Smith of Lebo, Kans., who was associated for many years with the State forces of Kansas. Dr. John Harris of Topeka, Kans., has given me invaluable aid and advice in consultation. All of these men gave me aid and advice when it was <u>most</u> needed.

Bringing ourselves to date--this question presents itself--can Tuberculosis of bovines or better still tuberculosis of farm animals be eradicated? I say, it can be--provided the proper approach be taken. I have every faith in our State-Federal regulatory forces, <u>when they are in full cooperation,</u> and have a definite objective. Our profession has never fallen down when it was working in unison and the objective was thoroughly understood.

Under the present methods, control is questionable--but much more so is eradication. In fact--given sufficient time--under our present diagnostic procedures, present liaison, and present professional attitudes, we could return to our disease status of the twenties. Any one making such a statement should have some suggested changes that would tend toward holding the disease in

abeyance and that when elaborated upon and prosecuted diligently would eventually eradicate it.

My first move would be to reduce or entirely eliminate the importance of the N. V. L.

Second, I would revive and emphasize the importance of the suspect.

Third, I would reinstill in the veterinarians of the U.S.A. the old confidence that once existed. There was no doubt that there was a disease to conquer, that we did have the tools to cope with it, and that it was their responsibility as a part of a team to eradicate this malady. Nor was there doubt that upon the shoulders of each team member was a load to carry, and that tuberculin testing of bovines was not a routine matter but rather a challenge to again subdue an enemy of the industry, an enemy supposedly at one time well along on the road toward eradication traveled by contagious pleura pneumonia, glanders, tick fever, and so on.

Fourth, every State would have a State or Federal or a State-Federal TB expert to work with the practicing veterinarians as these men attempted to uncover the disease in all farm animals.

Fifth, area TB testing as done today should be eliminated as rapidly as a better way of accrediting counties is devised, if reaccreditation is necessary and means much toward eradication of the disease.

Sixth, intensify the search for tubercular lesions on the killing floors and promote better lines of communication and investigation between the slaughtering establishments, the State-Federal sanitary officers, the practicing veterinarians, and the suspected farm premises.

Seventh, except in remote areas, have all TB testing done by local practicing veterinarians. All private tests to be paid by the owners. All forced testing, farm cleanups, and so forth, to be done at State-Federal expense.

Eighth, use every accredited veterinarian in the U.S.A. for this work who expresses by word and deed his willingness to cooperate. Drop immediately those who are unwilling to maintain a high standard of work.

Ninth, promote in all States pay schedules that will keep professional men interested. Have the accredited veterinarians who are carrying on this program of eradication as well compensated as are comparable professional groups handling public health affairs.

Tenth, the Agricultural Research Service of the U. S. Department of Agriculture must press for a tuberculin of greater selectivity. In this day of otics, mycins, proteins, hormone additives, implants, and so forth, it will undoubtedly require a much different product than one quite usable and satisfactory in the twenties.

Adding to suggested change number one, could we not have it understood between State-Federal forces, veterinarians, and owners that one or more clean retests are always in order and necessary? Then, if lesions have been found we write the owner that "Because of the findings of open lesions [or some other description] two or more clean tests are necessary." I believe the reversal of procedure in this case would prove to be good public relations, and would prevent that oft repeated remark, "I knew all the time, Doc., that she was all right."

Adding to number two, if we are to eradicate this disease, we must dispose satisfactorily of each and every animal demonstrating deviations from the normal at the site of injection. Also, the problem herd must not be needlessly slaughtered, but must nevertheless be proven clean before releasing. Suspects should be designated by ear-tag numbers and quarantined to the farm until their status is proven. Allow them to move only for slaughter by official permit. Even though their moving is by permit, slaughtering should be authorized only in plants having full-time State or Federal inspection, with each case as a special and handled accordingly. It's surprising, the education a veterinarian receives in cleaning up these problem cases or herds. The industry loves to see the status of these animals <u>carefully</u> determined. Many problem herds in our practice have been remedied and they are going concerns today. They could have been completely wiped out at one time with false reactions.

May I add to number three that tuberculin testing in many instances today is being considered by veterinarians and owners as a routine matter to satisfy a city ordinance or a formality to reaccredit a county.

Adding to my fourth suggestion, permit me to suggest that if we are to try a new approach to this subject, let's elevate it to the importance it merits. Each State may well have a full-time TB expert, working nearly full time in the field with practicing veterinarians and their problem cases. These experts should be schooled regularly, regionally, nationally, and internationally, by the best brains in the game. Keep them well posted on all experimental progress with newer types of tuberculin, techniques, epidemiological findings, and so on. By all means encourage and provide a method to readily relay ideas, experiments, suggestions, and so on, from the field to Beltsville or some other Federal TB experimental point. <u>Solutions to our problems may come from the field rather than from the laboratory</u>. Provide one or more competent practitioners in each State to pinch-hit so that the men in the field are never left in trouble without a consultant. Thereby, we may prevent a young practitioner from being discouraged or a cooperative owner from losing faith.

Commenting on suggestions five and six, perhaps rather than spending our meager State and Federal funds on routine area testing, we should be improving our lines of communication and identification from the killing floors to the farm.

As previously stated in my seventh suggestion, all private tuberculin tests should be paid by those requesting the service. If suspects

or reactors are found or killing-floor findings point to certain herds, these areas should be handled with State-Federal funds by the practicing veterinarian in whose territory suspicion points. As we approach the time when large animal practitioners in many areas must be subsidized in some way if they are to stay, State-Federal funds for TB eradication can well be spent with them. Veterinarians who would have no part of area testing will take readily to a farm cleanup. It's professional; it's a challenge.

In number eight, I advocated using every available, willing, accredited veterinarian. He need not be a "Brain" nor have been a whirlwind Grade A student. A personal, burning interest in the subject of tuberculosis will overcome any lack of knowledge. "Know-How" is sure to follow.

Adding to number nine, the matter of being well compensated creates a better quality of work. Low pay will attract the lower quality group. We must have the very best men, in each and every State, engaged in this eradication program as well as the others.

In number ten, I made reference to tuberculin of the past, present, and future. While on this subject let us look for a moment at our progress with bovine brucellosis eradication. In addition to skilled laboratory technicians, we have a near-perfect antigen. Witness the respect held for our brucellosis test, both by the profession and the industry. In this matter of tuberculin testing we have an example of "a good workman requires good tools." No matter how high the quality of the testing agent, if it is improperly or carelessly used the results will be unsatisfactory. Likewise, the most skilled, conscientious veterinarian will soon tire and become discouraged if the testing material does not measure up to the usage required of it. Epidemiological studies must be pressed to determine the extent that our present livestock situation is a public health matter of pressing importance. Upon the results will depend to some extent the availability of appropriated State and Federal funds, also whether the matter at hand is more economic than public health and vice versa. We veterinarians on the farm can more readily sell a public health program than one purely economic.

Before closing, may I again refer to my fourth suggestion. I have already mentioned channeling information from the farm to the laboratory. Proceeding farther in this trend of thought, wildlife studies, especially of an avian nature, may well be engaged in. I am led to believe that avain type tuberculosis is on the increase. In our practicing area, farm flocks of poultry running at large are becoming obsolete, and housed groups are scarce. Perhaps we should give deep consideration to pigeons, starlings, and the English Sparrow. We in our practice find forage, feedbunks, watering facilities, and so on thoroughly soiled and contaminated with feces and dead bodies of these pests.

With pleasure and pride, I make the statement that in my 39 years of general practice in Kansas, I have received full cooperation at all times from our Federal office, the Live Stock Sanitary Commissioners

Office, and the School of Veterinary Medicine at Kansas State University, in the carrying out of our Kansas TB program.

To my audience, may I thank you for your presence and interest. To the sponsors of this program, may I thank you for the opportunity to express my viewpoints. I hope that what has been expressed will in some measure assist us in solving another veterinary problem.

SIGNIFICANCE OF ACCREDITATION OF THE PRACTICING VETERINARIAN

F. J. Mulhern, Assistant Director, Animal Disease Eradication Division, ARS, USDA, Washington, D. C.

Since the theme for this afternoon's session is "Get Maximum Results from Tuberculin Tests," the topic that I have been assigned does carry a great deal of significance.

Before we get into the use of veterinarians in our specific programs, such as tuberculosis, first let's analyze why we use accredited veterinarians. Contrary to some beliefs, the regulations devised by our Division and State Departments of Agriculture are for <u>purposes of preventing the interstate spread of communicable diseases of animals and not merely to control the interstate movement of animals</u>. It is always amazing to me to learn that certain individuals figure that regulations are solely for redtape purposes or to place restrictions. Regulations are generally not too popular, as they are looked upon as infringing on rights, and we often see the same attitude develop as most of us take when a policeman blows his whistle at us. We find it difficult to convince some practitioners that each section of the regulations is designed to fill in certain gaps to prevent the spread of animal diseases. Consequently, it is on this basis that certain animal movements are not permitted. Many times a great deal of reliance is placed on the integrity of the individual making these inspections, and usually the regulations are just as effective as its <u>weakest</u> link; in some cases the inspection being provided by accredited veterinarians is just that.

It is the goal of regulatory officials to develop regulations that will produce the maximum disease control or eradication with minimum interference of trade being carried out by that segment of the livestock industry involved. In developing regulations, we begin by allowing certain movements if the animals are inspected and certified by full-time State or Federal employees. This is done because it is felt that there is a definite control over such employees provided action has to be taken for improper inspections. In order to allow the maximum movements of livestock, it is often learned that total full-time State and Federal employees are not adequate to meet the demands involved in the movement of the animals. In such cases, the regulations provide that inspections can also be provided by "accredited veterinarians." It specifies "accredited veterinarians" because it was felt that the State and Federal regulatory officials have some control over them since their accreditation was provided by these same officials.

The main point that I am trying to get across is that the term "accredited veterinarian" is used or designated in our regulations not solely because they are veterinarians, but because they are veterinarians who can be relied upon to carry out the responsibilities necessary to prevent the interstate spread of animal diseases and because, when they don't, action can be taken to correct the situation. Some individuals ridicule our insistence that inspection testing or vaccination be done by these accredited veterinarians. When they state instances where certificates were issued and the vet didn't even see the animals, or when he merely glanced at the animals instead of carefully palpating the reaction when reading tuberculin tests, we see how vulnerable a position we are placed in when we insist that veterinarians perform these professional duties. From a public relations standpoint, the profession had better realize that this matter needs to be given full attention, and I feel that we as regulatory officials should carry this message to the veterinary profession.

What do we expect of the accredited veterinarian employed in a State-Federal tuberculosis eradication program? If all we needed was a person to inject tuberculin into an animal, this could be taught to any layman within a short period of time. We know that we need a person who has been trained and has a comprehensive knowledge of the disease so that he will give the owner reliable information; an owner particularly needs such a person when he learns that he has tuberculosis in his herd. With the extensive training given to veterinarians, he should have a broad knowledge relative to the allergic reactions produced by the tuberculin and recognize the need for proper technique. He has comprehensive understanding of the seriousness of tuberculosis as a disease and the devious ways in which the organism tends to perpetuate itself both within and outside the body. He recognizes the importance of keeping areas, established as free of the disease, from being invaded by infected and exposed animals; he therefore is very cautious in his testing of animals being shipped into such an area.

Well, now let's see what experience we have been encountering in using accredited veterinarians. In the majority of cases the accredited veterinarian is doing a commendable job, but there have also been instances that occur too frequently that seem almost unbelievable. We have found some who, after inoculating the animals, have asked the owners to observe them and report any changes at the site of inoculation; some who have injected the animals but did not return to examine the site of inoculation; some who have returned to examine the site of injection but ignored reactions that were quite evident, speculating that they were due to causes other than tuberculosis; veterinarians who have signed certificates that they have actually tested the animals when someone else has done it for them; and others who have admitted that their negligence was due to the fact that they had never observed a true tuberculosis reaction and wouldn't recognize it if it were there.

Let's review the history of the process of accrediting veterinarians in this country. For approximately a 30-year period an examination was

given to every veterinarian who requested it. Provided that he passed the examination and had a license to practice within the State, and further provided that his accreditation was recommended by the Federal Veterinarian in Charge and the State veterinarian, the accreditation was granted. Down through the years these examinations reached a point where no one failed and consequently very little prestige or importance was given to the exam. Later the examination was dropped entirely.

Only during the past 3 or 4 years have we started to give the test again. During the past 4 or 5 years a different approach has been taken toward the importance of accreditation, and as a result it has become necessary to remove accreditation from many more veterinarians than we like to mention.

Up to this point it seems probably as though all of us in this room are in agreement that the veterinary practitioner is at fault and we have picked him apart. However, if we are honest with ourselves, we can see that we have contributed to the problem.

First, what have we done to point out to the veterinary colleges that students were not being given adequate instruction on their responsibilities in the field of regulatory medicine? Second, what have we done to see that when a practitioner goes into practice and participates in our programs he is fully aware of his responsibilities and what we expect of him? Third, what have we done to convince him of the need for proper technique that we consider so important in the eradication of tuberculosis? Let's see what we have done about the problem.

During recent years at veterinary short courses and at meetings of State veterinary associations, animals have been sensitized with tuberculin and then tested so that all veterinarians attending could palpate the reactions and thereby become aware of reactions to tuberculosis.

Many conferences have been held with different veterinary associations explaining the seriousness of faulty techniques in contributing to bovine tuberculosis problems. In some States area supervisors spend 2 weeks to a month with practitioners when they have herds to test, until the regulatory official is satisfied that the practitioner has proper technique and confidence in his testing ability. Periodically the regulatory veterinarian rides with the practitioner to see that he hasn't deviated from the techniques previously given. Some regulatory officials don't like to do this, as they believe it creates hard feelings on the part of the practitioner; but if this is the result, we have failed in our public relations with the practitioner. Tuberculosis is a serious disease, and if we are not making progress in disease reduction because of faulty technique, which, then, is worse--lose the friendship of a practitioner or the confidence of the industry?

Veterinary schools have become much more interested in tuberculosis eradication, as is evidenced by two universities requesting funds for bovine tuberculosis research. We find regulatory officials getting opportunities to speak to veterinary classes on tuberculosis.

All of us should not miss an opportunity to present the tuberculosis problem to practicing veterinarians. Certainly, if they are exposed to it often enough to recognize the complex situation that has developed in our program owing to avian tuberculosis, human tuberculosis, Johne's diseases, and other acid-fast organisms, it will give then a better appreciation of the problem and undoubtedly create a more cooperative attitude with us. It seems that from a public relations standpoint we could spend some time very profitably explaining to them what we expect in the way of their services and the importance of certificates.

In conclusion, what can you and I as regulatory officials do to help this situation?

1. If there is a veterinary college within your State, make periodic contacts with the dean and members of the faculty and review the tuberculosis eradication problems with them. Stress the importance of graduates being fully informed on techniques and importance of the part played by accredited veterinarians in our regulatory programs. See if you can get State and Federal personnel onto programs to address and give testing demonstrations to students and practitioners on the importance of the veterinarians in different fields working together in order to further reduce the incidence of the disease.

2. See that we properly indoctrinate all accredited veterinarians on technique and their responsibilities in our type work.

3. Properly supervise new workers by accompanying them on their initial testing until we are satisfied that they have demonstrated proper technique. Ride with them periodically to see that they are applying the techniques.

4. Give warning when practitioners fail to handle their responsibilities, and if an infraction occurs again, recommend their removal from accreditation.

5. Place importance on the "accreditation" so that they will consider it in the same category as their license. Remember, if practitioners do not appreciate the importance and significance of accreditation, it is no one's fault but our own.

MAXIMUM RESULTS FROM COUNTY REACCREDITATION

R. L. Knudson, Assistant Director, North Central Area, Animal Disease Eradication Division, ARS, USDA, Washington, D. C.

The following was from the Report of the 22d Annual Meeting, United States Livestock Sanitary Association, "Tuberculosis Eradication" by Dr. John A. Kiernan. The date of the report was December 2, 1918. We might all consider at this time the appropriateness of his words in light of where we stand today:

"To be sure, sanguinity has filled none of us with more than a reasonable degree of confidence, but we have no room for pessimism and its devitalizing influence. Tuberculosis must be eradicated if it be possible to accomplish that result, and it is believed to be a possible achievement in view of our experience with the disease covering a period of years. We have too much at stake not to resort to every means that may be put into practice at any time, to extirpate this insidious foe. We are compelled to consider this disease from a national standpoint rather than from forty-eight sectional viewpoints. The live stock industry of each state is but a part of the agricultural resources of the nation....The deeper disease preys upon our cattle, be it in the North, South, East, or West, the lower will sink our resources. The live stock belongs to the individual, and our political system encourages the development of enterprise among its citizens. We may sell them, kill them, give them away, traffic and barter with them in any way we choose, just so long as they are all sound in health; but, should they, at any time, become diseased and be a source of danger to the stock of our neighbor, the law, which is made for all the people, restrains us under the police authorities of the state or federal governments, from jeopardizing the health of other animals."

On November 1, 1940, nationwide modified accredited status was reached. One of the basic procedures in connection with the program of eradication has been the periodic testing for reaccreditation of all of the counties in the country. The requirements for reaccreditation of counties are outlined; under the Uniform Methods and Rules which provide for a variety of ways in which this can be accomplished.

However, under the system of reaccreditation, evidence at hand indicates certain weaknesses which have developed. It was brought out by Dr. A. F. Ranney in his talk this morning on the status of tuberculosis eradication in the United States that confidence in our modified accredited areas is generally lacking. Might not this lack of confidence be chiefly due to the minimum efforts being made in reaccreditation of counties.

The results from the accreditation status could be evaluated with two different approaches; namely, minimum and maximum. The minimum results could come about following an accreditation procedure whereby the lowest number of cattle would be tested in relation to the infection disclosed so as to allow reaccreditation. This accreditation status would look well on the records, but if in doing this, the foci of infection were allowed to remain, it could be best described in the words of a bacteriology professor I had in school, "It ain't no good, no way, no how."

In comparison, maximum results that could be obtained from county reaccreditation might be summed up in that insofar as possible, it has been determined that all foci of infection have been located and proper disease control methods have been established. This type of approach might not be differentiated from the minimum approach as far as records

are concerned. In fact, analysis might even make it look worse because of the high number of reactors disclosed.

But it is hoped that we will all agree that in the long pull, maximum effort certainly will be needed to pave the way for eradication and will be more of a guarantee to the livestock owner of freedom from exposure, lessening the human health hazard, and more firmly establishing a true meaning to reaccreditation.

III. A BETTER KNOWLEDGE OF TECHNICAL ASPECTS

THE BACTERIOLOGY OF TUBERCULOSIS

David T. Berman, Department of Veterinary Science,
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When the program for this meeting was arranged, I was chosen to speak on the topic, "The Bacteriology of Tuberculosis." The arrangers then made my task impossible by allotting 20 minutes for my discussion. I interpret this as the grant to me of carte blanche to speak about those things which interest me most.

The tubercle bacilli are members of a fascinating group of organisms. They have the ability to grow from relatively large inocula in simple synthetic media consisting of an inorganic nitrogen source or an amino acid, a little glycerol and dextrose as a source of carbon, as well as a few mineral salts. They convert these simple substances into large masses of bacterial protoplasm. Some 40 or 45 percent of the weight of these bacilli is of lipids, including some of unique chemical structure not found elsewhere in nature.

These lipids have exceptional properties. They are clearly associated with the acid-fast staining character of the bacilli; they are associated as well with the nature of the lesion in tuberculosis and with the development of the delayed hypersensitivity with which we deal in the tuberculin test. This directive attribute of the tuberculo-lipids can be illustrated in a rather simple experiment. If animals are injected (sensitized) with tuberculo-protein, they will develop an immediate hypersensitivity to that protein. This hypersensitivity can then be demonstrated by a cutaneous test in which the reaction will develop within a matter of minutes after administration of the same protein. If the protein is administered systemically, the immediate hypersensitivity will be seen as the typical anaphylactic reaction. If the animal is injected (sensitized) with the same protein plus the extracted lipids from tubercle bacilli, the delayed hypersensitivity reation will be stimulated. In this reaction if the protein is administered as a cutaneous test reagent, the reponse does not become apparent until after the passage of some time--usually more than 8 hours, and does not reach peak intensity until 48 hours or more. The influence

of the tuberculo-lipid can be shown even more dramatically by using it with some nontuberculo protein--say egg albumin. In such an experimental situation the animals will develop delayed hypersensitivity of the tuberculin type to the egg albumin antigen.

I should like now to consider the classification of tubercle bacilli; and to do this, it seems to me, we should first consider the basis of our present classification of bacteria. If we examine this classification closely, we find that it is a grouping of organisms on the basis of similarities; thus, we group into a genus - Mycobacterium, a group of bacteria which can be stained with difficulty but which retain stains when treated with acidified alcohol. Within the genus we recognize species as natural groups which can be distinguished on the basis of their having more characteristics in common than ones in which they differ. That is, if we take 100 cultures of Mycobacterium which have been isolated from cows and 100 which have been isolated from chickens and 100 which have been isolated from human beings and ask these organisms the right questions, we will find a vast majority will arrange themselves into 3 groups. The seventh edition of Bergey's "Manual of Determinative Bacteriology" gives these groups species designation: Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium avium.

What kinds of questions might we ask? We might investigate the temperature range over which they will grow, their rate of growth, the effect of glycerol on the growth, the relative virulence for experimental animal such as mice, guinea pigs, rabbits, and chickens. Let us see how our three species group themselves:

			Relative virulence for —			
	Growth at 37°C.	Effect of glycerol	Mouse	Guinea pig	Rabbit	Chicken
M. tuberculosis	++	Growth enhanced	++++	++++	+	0
M. bovis	+	Growth not enhanced	++++	++++	++++	0
M. avium	+++	Growth enhanced	±	0	+++	++++

Not all strains will adhere to each of these characteristics. For example, it has been found that strains of Mycobacterium bovis grown on culture media for many transfers may develop the capacity of growing more rapidly than the typical strains.

There are other organisms in these natural groups which we call species within the genus Mycobacterium. Bergey's seventh edition recognizes two saprophytic species which grow at 37°C., and one parasitic species which grows at 33°C. In addition there are recognized M. paratuberculosis, about which Dr. Larsen will speak, and the human and rat leprosy bacilli.

Many strains of these have been isolated from skin lesions or circumscribed lesions of lymph nodes of a variety of animals. When we ask these cultures the questions we asked of our organisms isolated from cases of tuberculosis, we find that they all behave more or less alike, that is, like the saprophytes found in soil and water. They will not kill guinea pigs or chickens as do those isolated from obvious cases of tuberculosis. In addition to those organisms so well characterized that they have been given species names, there is a group of so-called anonymous acid-fast bacteria, which have been isolated from pathogenic processes in man and other animals and about which not enough information is available to determine their place in our scheme of classification.

The few characteristics which I have cited are far from the number which have been examined by various investigators. Such things as the chemical characterization of the extracted lipids, enzymatic activity of intact cells, serological studies of proteins and lipo-poly saccharides and others have been investigated. As with those characteristics first described, when large numbers of strains are examined in a large number of tests, the strains arrange themselves into natural groups on the basis of similarity; but certain of the characteristics will overlap. One of these overlapping characteristics which is of decisive importance to those of us concerned with eradication of bovine tuberculosis is the cross-reactive serological specificity of proteins. This in turn is expressed as cross-reactivity in tuberculin tests, a subject which will be discussed by Dr. Karlson.

One final point which I should like to make in this brief discussion is that a relatively small amount of information is available on the bacteriology of bovine tuberculosis. By this I mean that relatively little taxonomic investigation has been made of the organisms isolated from tuberculous lesions from the many animals which have been slaughtered in regular tuberculosis control programs. I should like to present to you the results of one such investigation by Plum of Denmark in which 1,264 cultures isolated from various types of lesions found in tuberculin-positive animals were actually speciated.

Source of Organisms	M. Bovis	M. Avium
	Number	Number
Lung or pulmonary lymph nodes	769	24
Mesenteric lymph nodes	109	241
Retro pharyngeal lymph nodes	94	27

These data make it abundantly clear that organisms other than *Mycobacterium bovis* may produce tuberculin sensitization and even produce lesions detectable upon post-mortem examination. I suggest to you that these findings are not likely to be unique for cattle in Denmark, and I wish to submit that their relevance for our tuberculosis eradication program must be considered. If this type of tuberculous infection is to be eradicated, then attention must also be directed to the sources of infection other than direct bovine to bovine transmission.

THE PATHOLOGY OF TUBERCULOSIS

M. J. Twiehaus, Department of Pathology, Kansas State University of Agriculture and Applied Science, Manhattan

Two topics on this morning's program might well be described as The Causes and Seats of Disease and should certainly add to better knowledge of the technical aspects of tuberculosis. Dr. Berman has just finished discussing the bacteriology of tuberculosis; my subject is the pathology of tuberculosis.

Tuberculosis generally may be defined as a chronic infectious disease of animals and man caused by the Mycobacterium tuberculosis organisms and characterized by the formation of small nonvascular nodules or tubercles which have a tendency to undergo caseation and necrosis. It may be acute in some cases. The tubercle bacilli enter the body through any of the natural body openings or through wounds in the skin. There is no doubt but that most infections occur by way of the alimentary tract or the respiratory tract, depending upon the species. (NOTE: Swine and poultry infected chiefly by alimentary tract; cattle infected probably by respiratory and digestive tracts.).

The three types of Mycobacterium tuberculosis organisms--human, bovine, and avain--produce similar lesions closely resembling one another morphologically. In our food producing animals, the bovine type of mycobacteria causes tuberculosis in bovines, although sensitization to human and avian types may occur. The avian type affects poultry; the bovine, avian, and human types are pathogenic for swine; and sheep are only slightly susceptible to the bovine and avian types. The lesions, or tubercles, may be found in any of the tissues of the body. Concerning food-producing animals, the tubercles in cattle are most frequently found in the lung, pleura, liver, spleen, and especially the peritoneum. Regional lymph nodes are also frequently involved. In swine, lesions most frequently are localized in the cervical, bronchial, mediastinal, and mesenteric lymph nodes. The involvement of the liver, spleen, and mesentery is more common in swine than in cattle. It should be remembered that lesions may occur in any of the organs or lymph glands. In sheep, when infections occur, the lesions are generally confined to the thoracic cavity or respiratory tract and, in some cases, may be generalized. In poultry, the lesions are found commonly in the liver, spleen, intestines, lungs, bones, joints, peritoneum, kidney, and so fouth. Lymph nodes are not so numerous in birds and are, therefore, less important as far as tuberculosis lesions are concerned.

Concerning non-food-producing animals, the dog and cat are susceptible to both the human and bovine types, and the lesions are confined chiefly to the lungs, pleura, and intestines. The adjacent nodes may or may not be involved.

The gross appearance of the tubercle is that of a hard, firm, white-gray or yellow nodule, depending, of course, upon the species.

In the bovine the tubercle, upon incision, appears as a yellowish, caseous, necrotic mass. The center is dry and firm whereas an abscess is soft and contains pus. The connective tissue capsule is usually thick and dense and multinucleated giant cells are numerous, but lymphocytes are few in number. Calcification is common in the bovine and in such the tubercles or nodes, when incised, exert a grating sound indicating the presence of calcarious material. (NOTE: All calcarious lesions are not tuberculosis tubercles.) On the serous surfaces, tubercles appear as firm, dense, pearly like nodules frequently referred to as "pearly disease." In swine, the tubercle, when due to the avian type, is less likely to show calcification and caseation than when it is due to the bovine type. When present in other organs, particularly the spleen, it may assume a neoplastic appearance.

In fowl, the tubercle shows extensive caseation usually without calcification. The connective tissue capsule and cellular elements are usually sparse.

Tubercles develop when tubercle bacilli gain entrance into the body. They are usually immediately phagocytized by neutrophils and, therefore, may be localized or carried to nodes or other areas of the body. These neutrophils apparently are not capable of destroying acid-fast bacilli. In fact, there is some evidence that these bacilli are highly toxic to those cells. The death of the neutrophil evokes the accumulation of another type of cell, the monocyte or epitheloid or endotheloid cell. These epitheloid cells encircle and engulf the bacteria and the dead neutrophils. They apparently are not able to inhibit growth of the lesions. The Mycobacterium organisms apparently have the ability to multiply within these epitheloid cells and produce a toxic substance that causes the adjoining cells to undergo caseous necrosis and die. Therefore, more epitheloid cells are being laid down around the caseous mass. In the center of this tubercle, cells lose their outline, the nuclei disappear, and structural detail is lost. When stained, a homogenous center staining red with eosin is observed. These large mononuclear cells or epitheloid cells may form multinucleated giant cells (Langhans giant cells). These cells are formed by the fusion of a number of epitheloid cells. They may attain a great size and contain large numbers of nuclei, usually arranged either around the periphery or at one or both poles. Giant cells usually are not found until necrosis has occurred, and are found in small caseous areas or at the edge of larger areas. Giant cells are very characteristic of tuberculosis but are also present in other chronic inflammations. In acute cases, they may be nonexistent. The granulation tissue is usually surrounded by a zone of lymphocytes and young fibroblasts arranged diffusely or in clumps around the lesion, frequently in or near a blood vessel. As the lesions become older, they become encapsulated by connective tissue of varying thickness. This encapsulation with the central caseation creates the most characteristic histological feature of the tubercle. The fate of this tubercle is dependent upon the resistance of the host and the aggressiveness of the bacilli or virulence. The focus of the infection may pass through a cycle in which the initial area of destruction remains small, or the

tubercle remains localized owing to the "walling off" reaction and subsequently heals by progressive scarring followed many times by calcium deposition within the area of fibrosis. In case the resistance force is inadequate to control the infection, the destructive process may extend to other areas or other organs to cause death.

I should point out that other diseases and disease processes may produce lesions similar to the tubercle. Granulomatous reactions are also produced by actinomycosis, foreign bodies, fungi, and so forth. Calcification may occur in the center of this nodule, particularly if the species is bovine. This is not true in cases mentioned in avain tuberculosis. The young tubercle is relatively avascular in nature. This, along with the possible toxic action of the organism, is probably the cause of the central caseation necrosis that occurs. The pH of the tubercle lesion is such that it is unfavorable for the infiltration of polymorphonuclear neutrophils. Therefore, protolytic enzymes are not present for the digestion of the caseous necrotic mass in the tubercle. Secondary or daughter tubercles may arise from the primary or mother tubercle. This may occur by several ways. Phagocytes that have engulfed acid-fast organisms in the tissue surrounding the primary tubercle may migrate to other areas of the body.

The phagocytes, being unable to destroy the bacteria, are responsible for the spread of this infection or the formation of daughter tubercles. In this way, the infection may be carried to neighboring lymphatics, lymph nodes, and even to the bloodstream. Extension by the bloodstream occurs when the pathological process involves the wall of individual blood vessels and forms a connection between a caseous focus and the lumen of the vessel, or when a tubercle may develop in the vessel wall and the bacilli enter the bloodstream. In this case, the formation of numerous tubercles in the various organs occurs. Miliary tuberculosis results when masses of organisms invade the bloodstream. Bacilli may also enter the lymphatics from the parenchyma of the lungs and produce a diffuse fibrinous inflammation, resulting in nodular growths or pearl nodules. These nodules on the serosal surface may be pedunculated or firmly attached. They may coalesce or unite with each other to form cauliflower or grapelike growths. Infection may extend to the pericardium, resulting in a marked thickening of it. Adhesions and tubercles may develop in the heart wall.

Extension may also occur from the lung parenchyma when erosion of a tubercle into a bronchiole occurs. Drainage of the caseous focus into the bronchiole gives a direct communication with plenty of oxygen and the bacilli are favored by increased O_2 tension and removal of the acidity of the tubercle. Under these conditions, a cavity is produced that may involve the entire apex or lobe of a lung. The bacilli may disseminate through the airways to other areas of the lung or the upper respiratory tract, thereby contaminating the mouth and nostrils by this exudative material.

When lymph nodes contain tubercles, the initial change consists of a uniform pulpy enlargement and macular discoloration. The cut surface, at a later stage, will appear gray to red in color and may contain whitish or calcarious areas. In advanced cases, the gland may become greatly enlarged, be of firm consistency, and when incised be surrounded by a fibrous capsule and center portion consisting of a caseous or pasty mass. Greatly enlarged lymph glands may cause lateral displacement of the esophagus or compression of the trachea.

IN CONCLUSION:--the Basic Tissue Reactions in tuberculosis are:

1. When the tubercle bacilli become lodged in the body, they evoke a characteristic proliferative, cellular microscopic granuloma known as a tubercle.

2. The tubercle consists of a group of mononuclear cells that resemble epithelial cells and are, therefore, referred to as epitheloid cells.

3. In this nest of epitheloid cells or surrounding them are found large multinucleated giant cells (Langhans type).

4. Surrounding this group of cells is a zone of fibroblasts and lymphocytes.

5. In this tubercle, caseous necrosis occurs, and it is this change that creates the characteristic histologic feature of the tubercle.

PATHOLOGY AND THE DIFFERENTIAL DIAGNOSIS OF TUBERCULOSIS

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It is generally recognized by animal disease regulatory officials that the followup tuberculin testing of herds based on the detection of suspected tuberculous lesions in nontested cattle has proved to be a valuable adjunct to the tuberculosis eradication program. As a result of this procedure, nearly 15 percent of all tuberculin reactors are uncovered annually and new foci of infection are being discovered in unsuspected herds that might otherwise be a source of continued spread of the disease.

Unfortunately, not all infected animals found at slaughter are traceable to their source of origin, owing to inadequate means of identification. As improved methods are developed and more infected animals can be traced, the number of reactors will increase proportionately.

Prior to 1950 the followup testing of herds was pursued on the basis of the post-mortem diagnosis of tuberculosis; and not infrequently, particularly in range herds, the tuberculin test was negative. Such a practice is not without some chance of error, since there are a number of other bovine granulomas that are often grossly indistinguishable from tuberculosis.

In 1951 the Animal Disease Research Laboratory, Denver, Colo., of the Animal Disease and Parasite Research Division reported on a series of 77 visceral lesions of suspected tuberculosis found in nontested cattle which were submitted for laboratory examination. This study revealed that 53 percent of the lesions were granulomas arising from other causes. The diseases most frequently confused with tuberculosis were actinobacillosis, mycotic infections, Corynebacterium pyogenes infection, parasitic lesions, and carcinomatosis.

The observations of the study were in part responsible for the issuance of a memorandum by the Meat Inspection Division in 1950 requiring laboratory confirmation of all suspected lesions of tuberculosis found in nonreactor beef-type animals before action is taken by the Tuberculosis Eradication Section.

As a consequence of that memorandum, there was a marked increase in the number of lesions received for laboratory examination. While the majority of the specimens were from nontested beef-type animals, there were many lesions from dairy animals as well. This afforded an opportunity to examine an additional 418 cases received during the 4-year period 1952 to 1956. Table 1 presents the laboratory findings on the 418 bovine granulomas submitted of nontested animals.

Table 1.--Laboratory diagnoses of 418 lesions from non-tuberculin-tested cattle, submitted as suspected tuberculosis

	Cases	Percent		Cases	Percent
Tuberculosis	227	54.3	Mycoses	2/ 21	5.0
C. pyogenes	84	20.1	Parasitic	3/ 10	2.4
Actinobacillosis	49	11.7	Staphylococcus	4	1.0
Neoplasia	1/ 22	5.3	Streptococcus	1	.2

1/ Carcinomatosis, 21; mesothelioma of peritoneum, 1.
2/ Coccidioidomycosis, 12; mucormycosis, 4; nocardiosis 2; other, 3.
3/ Pentastomiasis, 9; degenerated echinococcus cyst of liver, 1.

An analysis of the laboratory findings as shown in the table revealed confirmation of the post-mortem diagnosis of tuberculosis in 227 cases (54.3 percent) as against the other diagnoses in 191 (45.7 percent). The same diseases showing gross similarity to tuberculosis were encountered in this series of cases as in the smaller group of 77 already mentioned.

In deference to the veterinary meat inspectors who submitted the specimens, the nontuberculous lesions were in most instances grossly indistinguishable from tuberculosis and quite properly had been forwarded for laboratory confirmation. Conversely, lesions from nine animals were submitted with some other diagnosis which on laboratory examination proved to be tuberculosis (seven generalized). Among these were carcinomatosis, four; actinobacillosis, two; coccidioidal granuloma, two; and undetermined mycotic granuloma, one. Such discrepancies might have been avoided if a diagnosis of tuberculosis had been given first consideration.

The post-mortem diagnosis of tuberculosis in the bovine is usually based on the yellowish appearance of a necrobiotic process whether the lesion is caseous, caseocalcareous, or calcified. This is a rather constant feature of a tuberculous lesion, and even the purulent exudate in a larger lesion undergoing liquifaction necrosis remains yellow.

In contrast, the purulent exudate of most of the other common bovine infectious granulomas has a greenish lustre which affords a basis for differentiation from a tuberculous process. Examples are the greenish viscid pus of actinobacillosis, actinomycosis, coccidioidomycosis, nocardiosis, mucormycosis, *C. pyogenes*, and the inspissated pus of pentastome foci in lymph nodes.

Cancerous tissue (carcinoma) is commonly yellowish and is not infrequently confused with tuberculosis, as indicated in the table. Metastatic lesions of carcinoma of the eye or the uterus may be mistaken for granulomatous tissue if the primary site is overlooked.

As the purulent content of the nontuberculous granulomas is replaced by granulation tissue, its greenish lustre gradually disappears, the lesions become a yellowish caseocalcareous or calcified type of tissue which has a strong resemblance to a tuberculous process. Even the greenish nodules in lymph nodes caused by migrating parasitic larvae may eventually turn gray or light yellow and be confused with tuberculosis.

Most of the common granulomas can be diagnosed by appropriately strained films, by wet press preparations, or by histological sections. Cultures or animal inoculations may be made for further confirmatory diagnosis if necessary.

The demonstration of acid-fast bacilli morphologically typical of Mycobacterium tuberculosis in smears or in sections of a granulomatous lesion is generally acceptable for the diagnosis of tuberculosis. The presence of well-defined rosette formations in unstained press preparations or tissue sections of a purulent or granulomatous lesion is indicative of either actinobacillosis or actinomycosis. Further differentiation of these entities can be made by stained smears, gram-negative coccobacillary organisms being diagnostic for actinobacillosis and gram-positive branching filamentous forms indicative of actinomycosis.

If spherules with a highly refractile double-contoured wall are seen in direct examination or on histological examination of a granulomatous lesion, a diagnosis of coccidioidomycosis can be made without additional laboratory tests. Likewise, a tentative diagnosis of mucormycosis or nocardiosis can sometimes be made by similar laboratory procedures; but the organisms are best seen in tissue sections, and this is perhaps a better means of diagnosis.

A diagnosis of Corynebacterium pyogenes infection can be made by the demonstration of short gram-positive pleomorphic bacilli in either stained smears or on histologic examination of the lesion. In older lesions the organisms may be sparse and difficult to find, and cultures are advisable if sufficient material is available.

It should be mentioned that nearly all the 84 lesions diagnosed as C. pyogenes were examined histologically in search for acid-fast bacilli, since we were unable to demonstrate such bacilli in stained smears. We likewise failed to find acid-fast bacilli in tissue sections after prolonged search despite the microscopic resemblance to a tuberculous process of some of the lesions.

In nearly all instances, however, residual foci of polymorphonuclear leucocytes were still apparent in the lesions, a feature not usually seen in an old tuberculous process. Whether some of these cases would have proved to be tuberculosis on culture or animal inoculation is problematic.

The most common parasitic condition encountered in this series causing confusion with tuberculosis is a lesion resulting from

migrating linguatula larvae. These are more often found in the mesenteric lymph nodes but are also occasionally seen in the thoracic chain of lymphatic glands. The larvae may also migrate into the mediastinum, where they produce a greenish serous exudate which may extend into the adjacent interlobular spaces of the lung.

The greenish foci or nodules are more often located at the periphery of the affected lymph nodes and result from a heavy infiltration of eosinophiles which represent a response to the dead and disintegrating parasites. Older lesions lose their greenish color, become gray or light yellow, and may undergo calcification so that they resemble a tuberculous lesion. The lesions are encapsulated and are easily shelled out, in contrast with the fixed tissues in a tubercle.

We have failed to demonstrate the larvae or residual fragments in any of the lymph node lesions, and in only one instance was an intact parasite seen in the greenish inflammatory exudate in the mediastinum. Nine of the parasitic granulomas in this series were diagnosed as "pentastomiasis," a term applied to this condition in nearly all meat inspection textbooks.

The other parasitic lesion shown in the table was a yellowish necrotic granular focus in the liver. There were no other visceral lesions in the carcass. Direct smears were negative for acid-fast bacilli. On histological examination the lamellar cuticle of a dead and disintegrating echinococcus cyst was still apparent in the necrotic debris.

Two of the four staphylococal abscesses involved the udder, and because of their yellowish granulomatous appearance tuberculosis was suspected. A pure culture of Staphylococcus aureus was recovered from both lesions. In tissue sections, clumps of gram-positive cocci were present within ill-defined rosettes which were contained in the purulent foci. This disease of the udder was formerly called botryomycosis, but is now designated "granulomatous staphylococal mastitis".

The laboratory findings in 418 bovine granulomas in nontested animals submitted with a suspected diagnosis of tuberculosis are presented.

The post-mortem diagnosis of tuberculosis was confirmed in 227 cases (54.3 percent) whereas 191 lesions (45.7 percent) were found to be other conditions.

The diseases most commonly confused with tuberculosis were caseo-calcareous or calcified lesions of actinobacillosis, coccidioidomycosis, mucormycosis, and *Corynebacterium pyogenes* infection.

Neoplasms, particularly carcinoma, are often confused with tuberculosis, and lesions produced by migrating pentastome larvae into lymphatic glands may be mistaken for tuberculosis.

The difference between the post-mortem and laboratory diagnoses warrant continued laboratory confirmation of suspected lesions of tuberculosis, particularly when the followup tuberculin testing of herds may depend on the diagnosis.

RECIPROCAL OR CROSS-SENSITIVITY REACTIONS TO TUBERCULIN IN CATTLE

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Some of the various procedures by which the diagnosis of an infectious disease is accomplished are as follows: 1, Isolation and identification of the specific micro-organism, 2, serologic tests for specific antibodies, and 3, tests for hypersensitivity to the etiologic agent in question. In the case of tuberculosis in cattle it is not practical to make bacteriologic examinations for tubercle bacilli, nor are there any dependable serologic tests for this disease. The tuberculin test, a test for hypersensitivity to tubercle bacilli, is at present the only reliable and practical diagnostic tool available for tuberculosis in cattle.

The value of tuberculin tests for bovine tuberculosis has been recognized for many decades. As early as 1892 Bang (1) 2/ reviewed the literature on the subject and cited his own experience to conclude that tuberculin was a useful agent for the control of tuberculosis in cattle. In 1914 McFadyean (2) wrote that the only satisfactory way of detecting tuberculous animals was by means of the tuberculin test. During the subsequent years it has been demonstrated repeatedly that bovine tuberculosis can be controlled by testing with tuberculin and eliminating the reactors. This has been the experience in the United States, Finland, Norway, Switzerland, Guernsey, and in some parts of Portugal (3). Where other methods have been tried, the disease has not been controlled (4).

1/ The Mayo Foundation, Rochester, Minnesota, is a part of the Graduate School of the University of Minnesota.
2/ Numbers in parentheses refer to Literature Cited, at end of this paper.

For tuberculosis in human beings also, the tuberculin test is accepted as a reliable and specific diagnostic test. Long (5) has written: "Few procedures employed in the campaign to eradicate tuberculosis have been of greater value than the tuberculin test. It has been useful in the diagnosis of tuberculosis in the individual patient and priceless in studying the epidemiology of the disease and laying foundations for public health practices in tuberculosis control."

Although reliance on the tuberculin test is based on its specificity, it is known that tubercle bacilli have antigenic components in common with other acid-fast bacteria (6). The problem of cross sensitivity to tuberculin will be the concern of the following remarks. I shall discuss briefly a few acid-fast bacteria which may sensitize cattle to tuberculin, giving rise to reciprocal sensitizations or cross reactions.

Mycobacterium avium 2/

Avian tuberculosis may be transmitted to cattle from infected poultry, swine, and wild birds (9). Avian tubercle bacilli have a relatively low virulence for the bovine species, and the lesions are usually small and localized. However, a few cases of generalized tuberculous disease due to avian tubercle bacilli have been seen in cattle (10, 11). The incidence of avian tuberculosis in cattle in this country is not known, but evidence has accumulated showing that it may be considerable. Van Es and Martin (12) demonstrated avian tubercle bacilli in 15 of 115 tuberculin reactors with tuberculous lesions. The remainder had bovine tubercle bacilli. Feldman and Karlson (13) isolated avian tubercle bacilli from 3 of 36 reactors, and McCarter and coworkers (14) found avian strains in 3 of 28 reactors.

In Denmark, Plum (15) examined a large number of specimens from reactor cattle by bacteriologic procedures and reported that of 2,286 specimens, there were 1,195 with bovine, 321 with avian, and 8 with human type tubercle bacilli. Plum suggested that many lesions in the 762 specimens with negative findings may have been due to avian infection, because they were predominant in the mesenteric nodes. Additional instances recorded in the review by Greuel (7) indicated that avian tuberculosis is not uncommon in cattle in various parts of the world.

The quantitative relationship between sensitivity due to avian and to mammalian tubercle bacilli is difficult to assess, because of variations in potency of tuberculin. However, using purified protein derivative (PPD) Green (16) found that in guinea pigs with experimental bovine tuberculosis it took 20 times as much avian PPD to give reactions comparable to those produced by mammalian PPD. This difference accounts for the relative specificity of avian and mammalian tuberculins in applying simultaneous tests with both tuberculins.

2/ An extensive review of the literature on avian tuberculosis in cattle is given by Greuel (7). The pathology of avian tuberculosis in cattle is described in detail by Feldman (8).

In cattle experimentally infected with avian tuberculosis Schalk and co-workers (17) did not find reactions to mammalian tuberculin, but this is at variance with the experience of most investigators (7). The extensive studies of Schalk and his colleagues showed that cattle infected with avian tubercle bacilli, either experimentally or by exposure to tuberculous poultry, would develop sensitivity to avian tuberculin. In general, however, such reactions did not persist when animals were removed from an infective environment. Plum (18) found that some cattle with avian tuberculosis reacted only to avian tuberculin but that often they reacted to bovine tuberculin as well. Usually, however, the reaction to avian tuberculin was greater than that to bovine tuberculin. In instances in which a clear-cut difference existed between reactions to avian and to bovine tuberculin, the type of bacilli isolated corresponded to the greater reaction.

The use and interpretation of the simultaneous tests in cattle are described by Francis (19) with the conclusion that such comparative tests are reliable in herds with few reactors. In badly infected herds, however, many animals have equal reactions to both tuberculins. Such animals should be removed, because many will be found to have extensive tuberculous disease due to the bovine type of bacillus. In relatively tuberculosis-free herds, reactions to avian tuberculin only should be considered as non-specific.

The relative specificity of avian and mammalian tuberculin in swine was recognized rather early. In 1925 Van Es (20) warned that if swine are tested with mammalian tuberculin only, some may escape detection. It was recommended that avian as well as mammalian tuberculin be used in swine. This is now the general practice. The literature on tuberculin tests in swine is reviewed by Francis (19) and by Karlson (21).

It is entirely possible that avian tubercle bacilli share antigenic components with a greater variety of acid-fast bacilli than do mammalian strains. The close relationship to Mycobacterium paratuberculosis in this regard is well known and is the basis for using avian tuberculin in the diagnosis of Johne's disease. Experimental animals injected with various Mycobacterium spp. may react to avian but not to mammalian tuberculin (22, 23). Of particular interest is the rather high incidence of reactions to avian tuberculin in the human population in certain areas (24-26), an incidence which is apparently not related to the incidence of avian tuberculosis in poultry. It is believed that in man a positive reaction to avian tuberculin does not necessarily indicate infection with avian tubercle bacilli (27).

Mycobacterium tuberculosis

Tuberculin reactions in cattle resulting from infection with human type tubercle bacilli are not cross reactions, nor can they be considered as nonspecific. The tuberculin now used in this country to test cattle is made from a human strain of tubercle bacillus. Also, there is practically complete cross sensitization between human and bovine tubercle bacilli.

However, infections in cattle with human type strains may be responsible for reactors in previously tuberculin-negative herds and in locations where bovine tuberculosis does not exist. This may be responsible for some so-called problem herds.

In general, cattle are quite resistant to human type tubercle bacilli. Usually any lesions produced by this strain are limited and tend to regress. The apparent low virulence of human tubercle bacilli for cattle led in early years to the use of virulent human strains in various kinds of vaccines for bovine tuberculosis (28). It was found that artificially infected cattle would react to tuberculin in 3 to 6 weeks and that most animals would become negative in 6 to 12 months. Because persistent lesions developed in a few animals and actually shed human type tubercle bacilli, such vaccines were discarded.

The role played by human type tubercle bacilli in creating problems in the control of bovine tuberculosis may be illustrated by citing several studies. In Finland, Stenius (29) noted a discrepancy between the percentage of reactors and the actual percentage of such cattle having lesions. A cooperative study between the Department of Agriculture and the Finnish Antituberculosis Association was established. When reactors were found in cattle, the physicians made thorough studies on all herdsmen, handlers, and anyone who had contact with the cattle. By 1938 they had examined 308 herds of which 60 percent were infected with open pulmonary tuberculosis by some person on the farm. A more recent study was made in Denmark (30) during the period from 1946 through 1948 on 915 farms where 3,075 reactor cattle had been found. Investigation showed that on 10.4 percent of the farms tuberculous persons were the source of infection, which accounted for 18.3 percent of the 3,075 bovine reactors. Further discussion on this subject may be found in the monograph by Sigurdsson (31) and in the detailed studies by Fromm and Wiesmann (32). The finding of tuberculin reactors among previously negative cattle may be a means of locating cases of tuberculosis in the human population (33,34). The occurrence of so-called false-positive reactions in cattle due to human tuberculosis suggests that the control of bovine tuberculosis depends on control of the disease in man (35).

Mycobacterium paratuberculosis 3/

The subject of paratuberculosis is discussed herein to emphasize the role played in sensitizing animals to mammalian tuberculin. The relationship of Johne's bacillus to tubercle bacilli is rather close with respect to cross-sensitivity reactions. Using PPD preparation, Green (16) found that it required only 10 times as much johnin as mammalian tuberculin to elicit the same reaction in guinea pigs with bovine tuberculosis. For avian infection it required only three times as much johnin PPD as avian PPD to get comparable reactions. This close relationship to avian tubercle bacilli is well known. The widespread occurrence of Johne's disease, and

3/ An extensive review on Johne's disease is given by Hole (36).

especially of the subclinical infections, makes it difficult to interpret the results of the avian tuberculin test in cattle. Schalk and coworkers (17) found that 15.6 percent of 507 cattle tested in stockyards reacted to avian tuberculin. This may not reflect the actual occurrence of avian tuberculosis in the group since some animals may have had Johne's disease. Smith (37) has reported examining various tissues from 100 apparently normal animals and finding Johne's bacillus in 7, avian tubercle bacilli in 5, and bovine strains in 3. He reported also that other data indicate that Johne's bacillus may be recovered from ileocaecal lymph nodes of 7 to 17 percent of normal cattle.

Various investigators have demonstrated that cattle with paratuberculosis may react to the routine test for tuberculosis. For example, Johnson (38) found that 17 percent of 175 "no-visible-lesion" reactors had Johne's disease as determined by examination of the intestinal tract, and an additional 7 percent of the group were suspects with regard to Johne's disease. Sikes and Groth (39) applied the tuberculin test to cattle with Johne's disease and concluded that many no-visible-lesion reactors are due to paratuberculosis. They found that when the animals were tested on the caudal fold 5.4 percent had typical reactions or were suspects, but that when they were tested on the neck 32 percent were classified as positive or suspect tuberculin reactors.

McIntosh and Konst (40) made a comparison of human and of bovine types of PPD and the respective old tuberculins in guinea pigs sensitized with avian and bovine tubercle bacilli and with Johne's bacillus. It was found that of the old tuberculin products the human type gave more and greater reactions in guinea pigs with avian and with Johne's bacillus infections. For the PPD products there was less difference, but the human PPD gave reactions about four times that of the bovine PPD.

Using cattle, Johnson and coworkers (41) sensitized five groups of animals with Johne's bacillus, human, bovine, and avian tubercle bacilli, and *Mycobacterium phlei*, respectively. The cattle were tested with seven different johnins and the homologous tuberculin. In cattle with avian tuberculosis, two of the johnins gave reactions comparable to the reaction caused by avian tuberculin. The results of the study indicated that avian tuberculosis and Johne's disease may be responsible for no-visible-lesion reactors but that *M. phlei* was not.

Of particular interest in the comparison of antigenic relationships between *Mycobacterium paratuberculosis* and tubercle bacilli is the lack of specificity in certain serologic tests. Larsen (42) quoted work done in Australia which showed that serum from cattle with Johne's disease gave positive reactions in the hemagglutinin test using cells sensitized with tuberculin. Sigurdsson (43) also recorded that the hemagglutinin test gives a cross reaction as do complement-fixation tests. Of interest is Sigurdsson's report that in guinea pigs Johne's bacillus produces antituberculosis immunity comparable to that of the BCG vaccine. Some work suggests that this may be true in cattle.

So-Called Skin Tuberculosis

The problem of so-called skin tuberculosis will be discussed only briefly as one of the causes of "nonspecific", or cross, reaction to tuberculin in cattle. The disease is characterized by the occurrence in the skin of firm or fluctuant nodules some of which may ulcerate. The lesions are seen often on the limbs, but in some cases they seem to follow the lymphatics up the lower part of the neck. Microscopically, they are granulomatous processes with varying amounts of necrosis, caseation, calcification, and many giant cells. Acid-fast bacteria are readily seen in most lesions. These have never been cultured on artificial medium in spite of many attempts. Experimental transmission of the lesion from animal to animal has not been accomplished according to the experience of most workers. However, Hedström (44) has reported success in inoculating three cattle with material from lesions rich in acid-fast bacilli. These animals became sensitive to avian and to mammalian tuberculin.

Hedström studied 606 specimens of so-called skin tuberculosis from 581 animals, each of which had reacted to the tuberculin test but most of which had come from previously tuberculin-negative herds. It was concluded that these lesions are not caused by any known acid-fast microorganism but that the unidentified micro-organisms seen in the disease process are the cause of the disease and are responsible for the sensitization to tuberculin.

Nocardia

The acid-fast members of the genus *Nocardia* present a peculiar problem in the differential diagnosis of tuberculosis. In culture they break up to form short acid-fast rods. Some produce soft, smooth colonies like those of avian tubercle bacilli. Unless we examine them properly they easily may be mistaken for *Mycobacteria*. A second point of confusion is that the appearance of lesions may be so like that of tuberculosis that histopathologic differentiation is not possible. The lesions contain acid-fast rods. These are epithelioid cells, caseation, and fibrosis.

A third point of similarity in some cases is the cross sensitization with tuberculin. It was found by animal experimentation that certain species of *Nocardia* may sensitize guinea pigs to tuberculin. In addition, tuberculous animals may be sensitized to filtrates of *Nocardia* cultures, that is, nocardin (45). From Africa (46), where bovine farcy or nocardiosis exists, it was recently reported that of 18 tuberculin reactors in a herd of 90 cows, 3 were found by culture to have *Nocardia farcinica*. It was found that cultures of *Nocardia* from these animals would sensitize guinea pigs to mammalian tuberculin. In experimentally infected calves there were reactions to mammalian as well as to avian tuberculin. However, these reactions varied in degree, and in some animals there was no (or only a transient) sensitivity to mammalian tuberculin. In experimentally infected calves, the tuberculin reactions did not persist after 72 days. It was recommended that where nocardiosis is known to exist, tuberculin reactors should be retested in 60 days.

How much of a problem this may be in America is not known. In the report of Feldman and Moses (47) describing their studies on 101 reactors from South Dakota, 16 animals were listed in which no tuberculous lesions could be found on microscopic examination but in which "ray fungi" were present. No tubercle bacilli, either avian or mammalian, were demonstrable. In a study of lesions from 31 reactors, Davis and Anderson (48) found that 11 had lesions other than tuberculosis. Some of these were recorded as actinobacillosis, as actinomycosis, and as fungous infection. The evidence that acid-fast nocardia may sensitize cattle to tuberculin is circumstantial, but the indications are that this should be considered in any study of the problem.

Other Mycobacterium spp.

In addition to the afore-mentioned micro-organisms, many acid-fast bacilli are known to sensitize experimental animals to tuberculin. In a review of the literature up to 1946 at least 25 presumably different kinds were listed as having some cross reaction with tuberculin (23). The significance of the pathogenicity of unidentified acid-fast bacteria in human infections is currently of great interest (49). Edwards and co-workers (25) have pointed out that different types of mycobacteria may be responsible for tuberculin reactions in people. It was found that among human beings, reactions to tuberculin prepared from various acid-fast bacteria were not uncommon. Of considerable interest is the finding that reactions to certain kinds of tuberculin are commoner in the Southeastern States, where, in addition, reactions to 250 tuberculin units (T.U.) but not to 5 T.U. of PPD are commonest. It is suggested that theses reactions are not due to tuberculosis but to some other mycobacterial species.

Some indication that there is a geographic difference in nonspecific reactions to tuberculin in cattle was recorded by McIntosh and Konst (40) in Canada and by Crawford (50) in the United States. The latter worker suggested that some acid-fast bacillus other that tubercle bacilli may be responsible for the geographic variation in the occurrence of atypical reactions to tuberculin. Crawford found that some mycobacteria could sensitize guinea pigs to tuberculin, but the reactions usually were small and not constant.

In Switzerland, Messerli (51) stated that atypical tuberculin reactions are seen more frequently in herds in upland pastures than in those in the lowlands. In studies on 364 cattle with atypical tuberculin reactions, Messerli found that 88 percent had greater reactions to a product called "rabin" (a tuberculin made from the Rabinowitch acid-fast bacterium) than to avian or to mammalian tuberculin. He recommended that cattle be tested with such a "tuberculin" in addition to avian and mammalian tuberculin and that when the reaction to it is greater, a nonspecific sensitization should be considered.

Hastings and co-workers (52) studied the problem of no-visible-lesion reactors and found that certain unidentified acid-fast bacteria from tissues of tuberculin reactors could sensitize cattle to tuberculin.

Karlson and Feldman (22) isolated an acid-fast micro-organism from tonsils of swine which sensitized guinea pigs to avian but not to mammalian tuberculin.

Unpublished data from laboratories at the Mayo Clinic show that various Mycobacterium spp. may sensitize experimental animals to avian but not to mammalian tuberculin, which suggests that, as mentioned previously, avian tuberculin may not be as specific as is mammalian.

The ubiquity of many kinds of acid-fast bacilli in nature requires caution in ascribing significance to them when they are found in animal tissues. This is particularly true when they are found in animals that have reacted to the tuberculin test. Such acid-fast bacilli from soil, water, feces, and forage may exist as incidental or transient inhabitants and have no significance with respect to the tuberculin test.

Comment and Recommendations

Although reciprocal or cross reactions with tuberculin are known to occur, the tuberculin test must be regarded as specific and reliable. Bovine tuberculosis can be controlled by a program of tuberculin testing and removal of all reactors. In 1916 in the United States 0.53 percent of slaughtered cattle were condemned because of tuberculous disease, but by 1959 the percentage was 0.000525, a thousandfold decrease (53). This was accomplished by depending on results of the tuberculin test in the detection and elimination of tuberculous cattle.

In a discussion on the tuberculin test in cattle in 1892, Bang (1) cited his experience in which he found that of 61 cattle with tuberculosis 58 had given a positive reaction to the test, while of the 16 with no lesions 13 showed a negative reaction and 3 had a doubtful reaction. This example, with others, led him to conclude that his results and those of others show that the tuberculin test, like many other things in this world, is not perfect. He stated also that the tuberculin test is more than 90 percent accurate, that it would be a great mistake to question this method just because it does not do everything we want, and that when examining animals at post-mortem one should not say that the disease is not present just because it cannot be seen. These remarks fit the present situation as well as being true decades ago.

Certain recommendations may be made:

1. All tuberculin reactor cattle should be thoroughly examined. A post-mortem examination must include more than the procedures designed for meat inspection. An examination should include bacteriologic studies to determine the type of tubercle bacilli. A program of control is on a firmer basis if the infection is known to be of avian, human, or bovine origin.

2. It should be determined if there is a significant geographic variation in incidence of tuberculin reactions in cattle. Should there

be such variation, attempts must be made to study soil mycobacteria and their presence in tissues of reactors as well as in normal cattle. Local wild animals should be studied as possible vectors of tuberculosis.

3. A practical and dependable serologic test for bovine tuberculosis is needed. Specimens of blood submitted for other examinations could be routinely tested for tuberculosis if such a test were available.

4. Liaison between veterinarians and public health officials may be of mutual aid in disclosing sources of infection. It should be routine procedure to examine all personnel in contact with reactor cattle.

5. Tuberculosis is a disease of many species. There is ample evidence that tuberculosis is transmissible from one species to another, whether it be human, bovine, or avian in origin. For control of the disease in one species it must be controlled in another. An educational program among public health workers, physicians, veterinarians, and livestock growers should emphasize that tuberculosis of man and animals has not yet been eliminated.

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SKIN LESIONS AND THEIR SIGNIFICANCE IN TUBERCULOSIS ERADICATION

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In the time available to me for this presentation, it will be necessary to adhere closely to the outline prepared, and I believe all that can be done is to present a summary of, and some comments on, the status of our thoughts and knowledge on skin lesions. I am especially pleased that on the panel and in the audience there are those who will not permit me to go too far astray and not permit me to leave you with any wrong impressions.

Name.--In 1916, 1919, and 1923, I reported on a lymphangitis in cattle caused by acid-alcohol-fast organism. In a fourth printed report in 1929 I suggested a new name: "Acid-fast skin infections of cattle." I purposely omitted any reference to tuberculosis in the name. During the interval between 1916 and 1929 and later the disease had been found in many parts of this country, and the reports on this condition appeared under various names, such as skin tuberculosis, tuberculosis cutis, subcutaneous tubercular nodules, skin lesions in tuberculin reacting cattle, subcutaneous tuberculoid lesions. When reports on the disease began appearing in foreign journals, other names were used in some instances. It really matters very little what name or names are used until the oneness or plurality of the acid-fast incitant of the conditions described under the various headings is satisfactorily agreed upon. For brevity and convenience "Skin Lesion" will be mostly used in this presentation.

Brief history and geographic distribution.--As already indicated, the disease was first reported in California in 1916 and since then

many reports from various parts of this country have appeared, especially in the twenties and thirties. In other countries the disease hadn't received much attention before the thirties. Sweden and Great Britain were among the first of European countries to study and report on skin lesions. From then on, many articles reporting and discussing the disease have appeared in Switzerland, Denmark, Holland, Norway, and other European countries. The latest European country to report the occurrence of this disease for the first time was Germany in 1951. Many of the European articles give excellent reviews of the literature and discussions and observation on skin lesions. I haven't observed any reports of the finding of this disease in France, nor has reference to such reports come to my notice. Perrard and Ramon in 1913, however, did describe briefly skin affection of cattle in France, which they called tuberculides. The condition described by them is generally considered to thave referred to the skin lesions.

Clinical appearance and pathology. --In brief, the disease manifests itself by the formation of nodular and corded masses along the course of the lymphatics. The seat of the trouble is usually one of the limbs. Most frequently the front ones have been found involved. In some cases lesions have been found in the abdominal and thoracic and neck regions. These nodules are mostly subcutaneous, but the disease process may penetrate the skin and discharge a creamy or glutinous pus often containing yellowish calcereous granules. The nodules vary in size from a pea to a goose egg. Frequently, a conglomerate mass of nodules are present involving areas of 200 to 400 sq. cm. In some cases the nodules are isolated; in other cases nodular masses are found from which corded paths of infection leading to other nodules could be traced. Upon palpation the nodules were either soft or hard, but upon section they were found to contain necrotic areas which in most instances had reached liquefaction stage.

In a few instances the organisms were plentiful; in the majority of cases, however, they were scarce. Organisms 3 to 5 microns (μ) long and beaded were found. Some were fine straight rods, 2 to 3.5 μ in length; others were slightly longer and slightly curved or bent. Organisms were found which were larger at one end. Coccoid- and kidney-shaped ones were also observed. The organisms retained the fuchsin stain when treated with acid or acid-alcohol. Occasionally smears showed scattering organisms which had given up the fuchsin and taken the counterstain.

Large numbers of guinea pigs and rabbits and a few chickens, mice, calves, and swine inoculated with material from the lesions failed to show lesions of diagnostic value even though smears showed a fair or large number of acid-fast organisms in the inoculum. In some instances the inoculated animals were allowed to live for 10 months after the inoculation; a good many were killed between 3, and 5 months. Rabbits and guinea pigs would occasionally show local lesions which disappeared within 2 or 3 weeks. Acid-fasts were sometimes found in these local lesions.

The British (Hole and Hulse) conveniently divide the lesions into three types as follows:

> "The macroscopic appearance of the sectioned lesions is variable and we now recognize three types; one or all types may be present in the same animal, but type 3 frequently occurs alone. In the first type the nodules are made up of living tissue, of firm consistency and characteristic light brown color, sometimes but not always showing small foci of necrosis. There always appears to be skin involvement with this type, careful sectioning revealing at least one spot in which the light brown tissue has replaced the normal white color of the dermis and reached and penetrated the epithelium. Type 2 is subcutaneous in position, and is formed of a tough capsule enclosing firm yellow granular-looking necrotic material, frequently quite dry and often gritty to the knife. The walls of these cavities may be thin and fibrous, or may show variable thickenings of the light brown tissue of type 1. Type 3 is the abscess type commonly seen on the hind legs, and is similar to type 2 except that the contents are fluid, creamy or creamy yellow pus of varying consistency. These abscesses always burst at some time in their development and discharge their contents to the surface. Afterwards more of the light brown tissue appears to develop, and the lesion becomes less prominent though more solid; pus, however, may form again and another discharge occur."

Adopting the British subdivision of types, Hedström in an extensive study on skin lesions in Sweden grouped the cases studied by him as follows: Type 1, 20 specimens; Type 2, 455 specimens, 20% of which were recorded as mild and 80% as severe; Type 3, 125 specimens which were considered severe. Only difference between this third type and severe cases of type 2 is that instead of caseated and calcified centers there are large cavities with yellow creamy contents mingled with calcified areas.

Microscopic appearance of skin lesions.--Considerable work has been done and written on the histology of skin lesions, and practically all reports agree that skin lesion sections show the tubercle structure, associated with tuberculosis; and I haven't encountered any report stating that the pathologist could distinguish histologically the lesion caused by *Mycobacterium tuberculosis* from that of skin lesion.

The subject of teat lesions should be briefly touched upon at this point. In Montana, Hadleigh Marsh in 1925 described and discussed teat lesion on cows, many of which reacted positively to tuberculin, but showed no other evidence of tuberculous infection. Teat lesions have

also been found by Day in Star Valley, Wyo., in 1921. For description we'll quote Marsh directly:

> "The lesions consist of one or more hard nodules, varying from 1 cm. to 5 cm. in diameter, situated just beneath the skin of the teat, usually in the proximal half. The lesion involves the skin, showing either an open sore, with a raw surface or scab, or scar tissue marking the healed ulcer. The milk duct was not involved in any of our cases, nor did the udder show any lesions of tuberculosis. The nodules consist of fibrous connective tissue, in which are imbedded small pus foci, varying in size from points up to about 2 cm. in diameter. These foci contain a smooth, yellow pus. In a few cases there were no definite abscesses, but strands of yellow tissue ran in an irregular manner through the connective tissue matrix. In several instances there was nothing apparent to the naked eye except a slight subcutaneous proliferation of fibrous connective tissue, which was slightly hemorrhagic.
>
> "Microscopic examination of these lesions, however, showed tubercle formation. None of the lesions showed any caseation, and there was no gross calcification, although sections showed some cacareous deposits."

In passing, your attention should be called to the observation that practically all articles discussing the pathology of skin lesions refer to yellowish creamy purulent material. In this connection you will recall that Dr. Davis emphasized that the pus in bovine tuberculosis is practically always yellowish in color.

You realize that not all "bumps" on and under the skin of cattle are skin lesions--and it is not rare that specimens thought to be skin lesions proved on laboratory examination or even on section in the field to be other forms of granuloma, fibromas, abscesses, or even demodectic involvement of the skin.

One of the differences usually mentioned between skin lesions and tuberculosis is that in the former there is no lymph node involvement but that in tuberculosis the lymph node is practically always tuberculous. I observed prescapular lymph node involvement in a few cases when the skin lesion extended close to that lymph node. It infrequently occurs. Experience of some of the European investigators has been the same. The lymph node lesions in skin lesion cases referred to here have proven by animal and culture inoculation not to be tuberculous.

Tuberculin allergy.--If it weren't for the fact that tuberculin allergy has been closely associated with acid-fast skin lesions, this disease would not be of any great importance. The disease has no apparent ill effect on the general health of the cattle, nor on their milk production, nor on their beef value. It is generally accepted that cattle with acid-fast skin lesions will show tuberculin sensitivity to the intradermal test in approximately 50% of the cases. Most investigators and reporters on skin lesions are of the opinion that the existence of such a condition in the absence of tuberculous infection can and does produce a tuberculin sensitivity sufficiently large in many cases to be definitely classified as reactors to the intradermal tuberculin test. Many of these investigators realize that this hasn't been experimentally proven. In order to present proof of this it would be necessary to isolate the organism and reproduce the disease by inoculation of such organisms, or at least reproduce the disease by direct inoculation of skin lesion material, into a susceptible animal and then demonstrate tuberculin sensitivity.

The difficulty with meeting these criteria has been that the causative agent has not been isolated and the disease hadn't been satisfactorily reproduced even by direct inoculation of skin lesion material. Hedström of Sweden, however, reported in 1949 the production of clinical skin lesion disease in cattle by the inoculation of skin lesion material and that such animals developed tuberculin allergy. He summerizes his findings on this point as follows:

> "One has, more and more, come to the conclusion that so-called skin tuberculosis is accompanied by allergy to tuberculin, but hitherto this has not been positively proved. In the inoculation experiments in which the author has succeeded in transmitting the disease to cattle, the experiment animals have shown allergy to bovine and avian tuberculin. There should hardly be any doubt that this allergy was a result of the inoculation disease. The author considers, therefore, that he has produced a definite proof that so-called skin tuberculosis is or, more correctly, can be the cause of allergy to tuberculin."

Allergy to tuberculin is definitely far less persistent in skin lesion cases than in tuberculosis. Tuberculin allergy has been considered by many to be of transient nature in skin lesion cases. The British with their single comparative cervical test find less frequent failure of tuberculin reactions on retest in skin lesion cases than we have experienced in the United States with our caudal test. Because of the difference in methods of testing in the two countries, comparisons cannot be satisfactorily made. Interestingly, they found some cases positive to mammalian tuberculin in the field which would be negative to this tuberculin but positive to avian tuberculin on retest in the laboratory.

Hedström reports as follows on the maximal period of tuberculin allergy in skin lesion cases in 74 herds in Sweden: 225, 1 year; 331, 2 years; 139, 3 years; 112, 4 years; and 19, 5 years.

Hedström also reports that in parallel investigations with avian and bovine tuberculins in skin lesion cases, he obtained as a rule a higher larger reaction figure for avian than for bovine tuberculin. This he claims is an aid in excluding bovine tuberculosis in herds showing tuberculin allergy. To some extent the British also make use of similar observations in their single comparative test, in which both avian and mammalian tuberculosins are used.

As already indicated, the intradermal tuberculin test will produce reactions in approximately 50% of visible skin lesion cases. It should be borne in mind that skin lesions have been present in reacting animals which were not found when a more careful examination would have readily detected such lesions.

Miscellaneous Observations and Comments

Some of the early reports on skin lesions maintained that the acid-fast organisms found in these lesions were true tubercle bacilli (*Mycobacterium tuberculosis*). It would take more time than should be devoted here to adequately discuss whether or not the lesion, the acid-fast organisms, and the tuberculin allergy associated with skin lesions are due to infection with tubercle bacilli or some other acid-fast organisms.

To assume that these are caused by tubercle bacilli, it must be assumed that the tubercle bacilli in skin lesions perish and cannot grow on suitable media, and cannot produce the disease in experimentally susceptible animals. The weight of clinical and experimental evidence does not support such assumptions.

It is universally recognized that tuberculosis of the skin can and does occur; such cases are rare and are usually reported in the literature. One can find such reports in German and no doubt in other journals published in the latter part of the last century and the early part of the 20th century.

It should be emphasized that when our reports or the reports from Great Britain, Sweden, or other countries indicate the absence of tuberculosis 1/ infection in the skin lesions or in the animals in which the skin lesions were found, such findings are based on more than ordinary routine inspection and laboratory work; in most cases practically everything was done on post mortem and in the laboratory to prove the existence of tuberculosis, short of sectioning the entire animal and inoculating every bit of tissue. Also in this connection it can be stated that the animals involved in most of the cases under discussion

1/ Here and elsewhere in this paper the word "tuberculous" refers to infection with tubercle bacilli.

in this paragraph, were from herds with histories which indicated freedom from tuberculous 1/ infection. Results of such efforts under such conditions deserve serious consideration.

The coexistence of skin lesions with tuberculosis in other parts of the body has been reported in this and in other countries, but such cases are rare. This leads to the following more or less related topic.

Skin lesions in badly infected tuberculous herds.--I was immensely interested, as no doubt most of you were, in Dr. Fincher's report this morning, of his observations on the TB situation in Greece. I was particularly interested in his failure to discover a single case of skin lesion upon careful examination of several hundred cattle in several herds which were badly infected with tuberculosis. Dr. Fincher's extensive contacts and his knowledge of both skin lesions and tuberculosis in cattle in New York State and his experience of many years in the clinical work of the Veterinary College at Cornell University give considerable weight to these observations.

During the course of many years I had the opportunity to observe and study skin lesions and tuberculosis in cattle in California. On several occasions I looked for skin lesions in herds that were badly tuberculous, without definitely locating any cases. I made a special effort to find skin lesions, without success, in a herd in San Mateo County, Calif., which was known to be badly infected with tuberculosis. In the same county, a short distance from this herd, we semiannually tested a herd of approximately twenty-five hundred cattle which we considered free of tuberculosis but in which there were many cases of skin lesion. Invariably at these semiannual tuberculin tests, one to two hundred intradermal tuberculin reactors were found, many with very strong reactions. Ten to twenty of the non-skin-lesion reactors were slaughtered after each test and examined under more than ordinary routine inspection and no tuberculous lesions were found. There may be some cross-immunity between skin lesions and tuberculosis. I am inclined to believe that tuberculosis would more likely protect against skin lesion than would the latter against tuberculosis.

Another thought in this connection, the history of reports of skin lesions in this and other countries seems to fit in with the above observation. We weren't bothered with skin lesions in this country until after our eradication program had materially reduced the number of tuberculous herds and animals. The same appears to have been the case with appearance of disease and reports of this disease in other countries 15 or more years after our first skin lesion report appeared, and their eradication program in many cases began in earnest that much later. It is reasonable to assume that our reports would have alerted the European veterinarians to hunt for skin lesions and that they would have detected and reported many cases providing such cases had been present to the extent that their later reports showed. It could, of course, be reasonably argued that failure to lesions in a small percentage of tuberculin reacting animals was not of very much concern

1/ Here and elsewhere in this paper the word "tuberculous" refers to infection with tubercle bacilli.

and that a search for an explanation of the failure to find lesions didn't especially rouse very much interest until after our eradication programs, and others, had made considerable headway.

Subcutaneous tuberculin test.--On the history and detection of skin lesion cases, the following observation should also be considered. Very few skin lesion cases, not more than 5%, will give positive reaction to the subcutaneous (thermal) tuberculin test. The introduction of the intradermal tuberculin test into the eradication programs may have had some bearing on directing attention to the presence of skin lesions.

Spontaneous absorption of skin lesions.--American and European veterinarians have noted spontaneous absorption of skin lesions. Andersson reports several such observations. Hole and Hulse report that in 33 skin lesion cases they observed 1 in which there was complete absorption of the lesion. More frequently, most observers have found a reduction in size and extent of lesions.

Occurrence of skin lesions in Switzerland has been reported largely in cattle of the Alpine regions. In Sweden it has been found in all counties, less prevalent around Malmo. In Great Britain it has not been limited to any section.

Acid-fast saprophytes and other mycobacteria which have been isolated from or associated with skin lesions have not, in gereral, been satisfactorily shown to include the etiological agent which could produce the kind of lesion and disease which we have been associating with skin lesions. This is a very general statement on this phase of skin lesions.

There are, however, some other points relating to the etiology of skin lesions which should at least be brought to your attention very briefly here. The vole bacillus (*Mycobacterium muris*), isolated in England from acid-fast infection of voles (field mice), because of its generally somewhat low pathogencity for guinea pigs and cattle and humans, received considerable attention as a possible agent to be used as a vaccine against tuberculosis in human beings and in cattle. Hole and Hulse injected material from a few cases of skin lesions into voles. They obtained completely negative results. Hedstrom reports some success in producing skin-lesion-like lesions in a few cattle by the injection of the vole bacillus. This was reported 12 years ago, and since no further report on this work has since appeared (at least none came to my attention), it may be assumed that these leads weren't very productive in helping clear the question of the etiology of skin lesions.

One more item. *Mycobacterium balnei* in Sweden and *M. ulcerans* in Australia have been proven to be the cause of human skin infections, and have been mentioned as possible clues to cattle skin lesions. These two organisms seem to have been differentiated from each other, although very similar in many respects. Just before I came east for this meeting, I received a publication which claims to have produced a skin-lesion-like disease in cattle by experimental inoculation of *M. ulcerans*.

The Australian workers (Tolhurst, Buckle, and Wellington) were unable to isolate *M. ulcerans* or any other acid-fast organisms from skin lesion cases in Australian cattle.

IV. ELIMINATING HUMAN EXPOSURE

THE PUBLIC HEALTH ASPECT OF BOVINE TUBERCULOSIS

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The Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, has launched and is prosecuting the most refined and far-reaching tuberculosis eradication program in the world. Dr. A. F. Ranney, Chief, Tuberculosis Eradication section, and his staff observed that although the incidence of cattle harboring tubercle bacilli has markedly decreased since the eradication program began 40 years ago, of the more than 4,096,909 reactors found to date, 69 percent were from 9 States and 24 percent from 13 other States. Thus, 93 percent of the reactors were found in 22 States. In the past 3 years the same 9 States provided 75 percent of the total reactors and an additional 11 percent came from the 13 States, making a total of 86 percent in the same 22 States. Tubercle bacilli reside mainly in the bodies of animals that react to the tuberculin test. Therefore, it is logical to exert special effort in the nine States where most of the tuberculin reactor cattle reside, while the usual program continues in the remaining States. Despite the fact that even in these States the percentage of tuberculin reactors is exceedingly small, it is the considered opinion of the Tuberculosis Eradication section that as long as there are tuberculin reactors there is a tuberculosis problem to solve. The solution consists of finding the sources of bacilli of the reactors and making sure that the bacilli in the reactor animals are not allowed to contaminate other animals and people.

With eradication of the last tubercle bacillus in mind, the Division is providing conferences to which some of the Nation's outstanding authorities on tuberculosis are invited. The first of these conferences, in many years, was held in East Lansing, Mich., in June 1958. From the standpoint of attendance and programs presented, these meetings have far surpassed all other tuberculosis, medical, and public health meetings in the United States. In the 1958 Conference 4 full days were devoted to concentrated programs designed to hasten the eradication of the tubercle bacillus. Speakers dealt only wih fundamentals including bacteriology, pathology, diagnosis, epidemiology, and prevention necessary to eradicate this organism. Tremendous emphasis was placed upon the tuberculin test as the most dependable diagnostic agent for the presence of disease in cattle as well as people.

This second conference, in Manhattan, has started in the same manner, and the remainder of the scheduled program carries the same fundamental subjects and the far-reaching vision which permeated the other conference.

These conferences demonstrate the perseverance and determination of the veterinary profession and its allies to reach a goal which was established more than 40 years ago. Throughout these years informed veterinarians have constantly kept their eyes on the goal. During and after 1892 they had accumulated such a store of knowledge through basic and applied research as to have complete confidence in their ability to eradicate the disease from cattle. When the program began in 1917 seasoned students of tuberculosis knew that eradication was far away. Dr. J. A. Kiernan (1917), first Chief of Tuberculosis Eradication Division, said, "There absolutely are no grounds upon which a reasonable estimate can be made of the number of years it will take to

eradicate this disease. All one can do is to make a guess as to the time, and it is my belief that if this nation succeeds in eradicating tuberculosis in fifty years, it will be one of the greatest heritages our successors will have handed down to them."

Although the goal has been attained in sizeable areas, there are still others where tubercle bacilli still lurk. Even in these areas the number of reactors is so small that one can safely predict ultimate success.

The world has never witnessed a finer demonstration of perseverance, patience, hard work, and complete confidence in attaining a goal than that exhibited by the veterinary profession and its allies in the effort to eradicate the tubercle bacillus. The recent conferences have convinced me that these fine qualities are still in vogue and there will be no turning back or even relaxation until the job is completed.

Bovine Type of Tuberculosis in Man Suspected

There is evidence that, in antiquity, there was fear of humans' developing disease from tuberculous animals. It was thought to be this disease that resulted in prohibition, included in the Mosaic law, of eating flesh from animals suffering from consumption. Dr. V. A. Moore pointed out that numerous enactments against eating of such flesh were contained in the Talmud, namely in the Mishnah codified at the close of the second century and the Gemara in the fifth century. All down through the centuries the danger of this disease in animals being transmitted to people is frequently referred to, including ninth-century church laws in Germany.

Carmichael, London, in 1810 believed cow milk was a frequent cause of scrofula in people. In 1846 in a 90-page book, Klencke presented clinical histories of 16 children who, having been fed on milk of scrofulous and tuberculous cows, all acquired tuberculosis. He concluded that scrofula in humans and the disease found in animals are identical. Cheauveau insisted that tuberculosis of cattle is transmissible to man through the use of meat and milk.

It was observed that tuberculosis frequently developed among men who took care of tuberculous cattle. In some villages where families lived in the stables in close contact with the cattle all winter, Beauce found a high prevalence of tuberculosis.

A large volume of literature accrued, and many veterinarians continued in their firm belief that tuberculosis in cattle is transmissible to people. When Koch announced the discovery of the tubercle bacillus in 1882, he assumed there was only one type; however, after Rivolta isolated the avian type of tubercle bacillus in 1890 and Smith the bovine type in 1898, the stage was set for actual typing of tubercle bacilli in the laboratory. Thus, it could be determined with accuracy whether the bacilli from lesions in people were of the bovine type.

Bovine Type of Tuberculosis in Man Proved

In 1902, Ravenel, employed by the Pennsylvania State Livestock Sanitary Board, was the first person in the world to present irrefutable

evidence that the bovine type of tubercle bacillus causes disease in man. From five cases he drew the following conclusion: "It is a fair assumption from evidence at hand and in the absence of evidence to the contrary that the bovine tubercle bacillus has a high degree of pathogenic power for man also, which is especially manifested in the earlier years of life."

Henceforth, the bovine type of tubercle bacilli were found in lesions of tuberculous people. For some time it was thought that people with the bovine type of tuberculosis had lesions only in the abdomen, because the bacilli were ingested, whereas those with tuberculosis caused by the human type of bacillus usually had lesions in the lungs, because bacilli were inhaled. Bitter controversies were waged over the ingestion and inhalation theories; however, these were resolved when Ravenel found that tubercle bacilli injected directly through the intestine wall into the lumen could soon be recovered from cycle on their way to the bloodstream. Krause injected tubercle bacilli subcutaneously in the inguinal region of animals and soon found them in the lungs and bronchial lymph nodes. Lemon and Feldman placed tubercle bacilli in pleural spaces of animals and soon found them deposited in various organs. Tubercle bacilli introduced into the trachea and bronchi may soon be found in other parts of the body. Therefore, location of lesions as a method of differentiating between those caused by the human and bovine types was abandoned.

In 1910, W. H. Park, bacteriologist, New York City Department of Health, estimated that on the basis of the prevalence of cattle type of tuberculosis among children about 10 percent of deaths among infants were due to the bovine type. He and Krumwiede made bacteriological studies and reviewed work of others involving 1,511 cases and found that 66 percent of the generalized tuberculosis in children was due to the bovine type of tubercle bacilli.

For example, Chang of Massachusetts (1933) found that in 200 cases of extrapulmonary tuberculosis the bovine type of bacillus was responsible in 71 percent of patients from 1 to 5 years and 11 percent in persons over 17 years. The average for all age periods was 27.5 percent. In 1937, Griffith, Oxford University, Cambridge, England, pointed out that in his country approximately 50 percent of lesions of the cervical lymph nodes and the skin, 25 percent of the cases of fatal meningitis, 20 percent of lesions in the bones and joints and genitourinary tract, and 1 to 6 percent of pulmonary lesions were caused by the bovine type of tubercle bacillus. It was estimated that in England about 6 percent of all deaths among humans from tuberculosis were caused by the bovine type and about 2,000 deaths occured annually, and at least 4,000 new cases of bovine tuberculosis developed each year among people.

Later Griffith and Munro investigated 6,963 cases of pulmonary tuberculosis in Great Britain and found that 241 of them were expectorating the bovine type of tubercle bacillus. The same year Cutbill and Lynn investigated 2,101 cases of pulmonary tuberculosis in a sanatorium and found that 2.28 percent were caused by this type of bacillus.

Hedvall, Lund, Sweden, reported 65 cases of pulmonary tuberculosis in man due to bovine type of tubercle bacilli. In 1942 he published a monograph reporting 94 persons with the bovine type of tuberculosis. He demonstrated that tuberculosis of bovine origin in man shows complete agreement with the corresponding forms due to the human type of bacillus. The only possibility of establishing the diagnosis is by typing the organisms, as had also been found by Griffith. Thus, the former belief that the bovine type of tubercle bacillus has a low virulence for man was untenable. Hedvall demonstrated that the bovine type of tubercle bacillus can be transmitted from cattle to man, from man to man, and from man back to cattle. To solve this problem he says it is imperative that the campaign against tuberculosis in cattle be carried on with greatest energy and that the goal must be the extermination of these infected animals.

In 1938 at the University of Toronto, Canada, among 500 tuberculous children, the bovine type of tubercle bacillus was found to be responsible in 9.6 percent. Organisms were found in numerous parts of the body of these children including lymph nodes, tonsils, adenoids, meninges, bones, joints, skin, kidneys and serous surfaces. These children came from places where the milk supply was not pasteurized and in every case they had consumed whole milk.

In the first few years of World War II there was a relative increase of 50 percent in tuberculous meningitis among children up to the age of 10 years in England. The Committee on Tuberculosis in war time of the British Research Council pointed out that city children previously supplied with pasteurized milk were evacuated to the country where they consumed raw milk. Examination of milk from individual herds showed that an average of over 6 percent of all farms were producing milk containing tubercle bacilli. Moreover, the bulk milk which was sold represented the mixed milk of 20 or more herds. Therefore nearly all bulk milk was contaminated with bovine type of bacilli. While not all of the neningitis was caused by the bovine type of bacillus, due weight was given to this organism as an important factor.

Holm says that in those parts of Denmark where tuberculosis among cattle has been highly prevalent nearly half of the cases of pulmonary tuberculosis in farmers have been shown to be produced by the bovine type of tubercle bacillus. He says further that most likely tuberculosis among cattle has been responsible for the fact that in some parts of Denmark tuberculosis morbidity and mortality were greater in the rural districts than in towns. Among 11,072 persons with pulmonary tuberculosis who were alive on January 1, 1944, cultivation only of tubercle bacilli revealed that about 3.5 percent had the bovine type. Among 138 patients of 70 years or over, 6.5 percent revealed this type of bacillus. From actual typings made in the Serum Institute in Copenhagen since 1932, among 18,231 cases of pulmonary tuberculosis bovine organisms were found in 14.1 percent, whereas among 4,186 cases of extrapulmonary disease the bovine type of bacillus was recovered in 19.8 percent. In this group the highest percentage, 34.2, was among those with cervical lymph node tuberculosis.

Tobiesen, Denmark, reported that he had seen 26 cases of pulmonary tuberculosis due to the bovine type of bacillus. Jensen of the same nation recovered the cattle type in stomach washings in 5 percent of 1,774 cases of pulmonary tuberculosis. In Holland, Ruys found this type of tubercle bacillus in 614 percent of 204 cases of pulmonary tuberculosis. Among stable boys and workers, Lang reported 20 percent of 40 cases were due to the bovine type.

In parts of the world where tuberculosis among cattle has not been well controlled the bovine type of tubercle bacillus continues to cause from 10 to 13 percent of the clinical disease among people.

The bovine type of tubercle bacillus on invading the tissues of previously uninfected persons produces the primary type of tuberculosis which results in sensitization of the tissues to tuberculoprotein. Therefore, when living bovine type of tubercle bacilli are present in the dairy products consumed by humans one finds a high incidence of tuberculin reactors among the children and young adults. As tuberculosis was controlled among the cattle herds county by county throughout the United States, there followed a sharp decline in the incidence of tuberculin reactors among those children born after the veterinarians' program became effective. H. R. Smith pointed out that in 1917 when the national eradication began, the death rate from extrathoracic tuberculosis was 22.5 per 100,000 whereas in 1942 it was only 3.5, a decrease of 84 percent.

As the disease was controlled in the cattle herds there also followed a definite decrease of pulmonary tuberculosis in man from a death rate of 124.6 in 1917 to 39.6 in 1942 and 7.8 in 1957. Thus, the control of tuberculosis in cattle, although not responsible for all of the decrease of the disease in man, has played an important role.

When the State Hospital for Crippled Children was established in St. Paul in 1897 and for some time thereafter, 50 percent or more of the admissions had tuberculosis of bones and joints. Now this disease exists in less than 1 percent of admission. The Medical Director, C. C. Chatterton, states that the reduction in tuberculosis cases became noticeable when pasteurization began to be effectively practiced and more so when the program for eradication of tuberculosis from cattle herds practically eliminated bovine bacilli infections in children and young adults. Apparently this was responsible for removing at least 20 percent of the bone and joint tuberculosis from the childhood population. The remaining 80 percent has for the most part been eliminated by protecting children against infections with the human type of organism.

Animals Contract Tuberculosis From People

Now that those working in human medicine have lagged 40 years behind the work of veterinarians, we are faced with the terrible accusation that cattle contract tuberculosis from people. Not so long ago veterinarians were criticized for not proceeding faster and preventing people from acquiring tuberculosis from cattle.

Since the veterinarian and his allies have so nearly eradicated tuberculosis from the cattle of the United States it is a rarity for a person to contract tuberculosis from a bovine. There is some evidence to justify the statement that now probably more cattle contract tuberculosis from people than vice versa. There is a strong likelihood that a sizable number of persons 25 years of age or older have this type of disease, contracted when bovine tuberculosis was still prevalent. Some of those persons now have or may later develop contagious tuberculosis from the descendants of the bacilli of the bovine type that were taken into their bodies many years ago. These organisms can readily produce the disease in cattle when persons who have them in their sputum or in opening sores are in contact with cattle or work with their feed and water. A dramatic case in point was observed by Tice. In 1929 a tuberculin retest conducted on a clean herd of cattle belonging to a milk peddler revealed that none of the adult animals reacted, but some of the calves did. The owner stated that he had been purchasing milk from an untested herd, separating it, selling the cream, and feeding the skim milk to his calves. He was informed he must stop buying milk from this source or the herd must be tested. There were 22 animals in this other herd, all of which reacted to tuberculin, and tubercle bacilli were present in the milk. When the animals were slaughtered and the carcasses examined, bovine type of bacilli were found in all of them.

The owner purchased new cattle, and periodic testing revealed no reactor until January 1942, when 5 of the 24 in the herd reacted to tuberculin. Two months later the remaining 19, plus 1 natural addition, were tested and 7 reacted. In April of the same year all of the 13 remaining cattle reacted. Thus between January and April 1942, the entire herd of 25 cattle had to be sacrificed because they reacted to tuberculin.

During the summer of 1942 the owner purchased 12 cattle which were nonreactors, but they became infected like the previous herd. A third herd was purchased and went the same way. Before June 1944, this man had acquired his fourth herd, which was lost in the same manner.

As a result of careless handling, this owner lost 97 head of cattle from tuberculosis. The owner then agreed to discontinue dairying and to have no more cattle brought upon his premises. He promised, furthermore, not to have contact with anyone else's cattle.

After each test of the four herds, all reactors were removed and promptly slaughtered. The stables were cleansed and disinfected and the sources of the additions were checked. In no case had the period between the acquisition of an animal and the time it reacted to tuberculin exceeded 6 months. After the slaughtering of four complete herds, the source of infection had not been determined. Doctor Tice carefully investigated all possibility of the animals having been infected from other animals but was unable to find such a source.

He learned that the owner of the cattle had been a patient in a tuberculosis sanatorium from March 12 to May 3, 1942. In November 1943,

cultures from the lesions of the cattle were typed at the New York State Veterinary College and at Homer Folks Tuberculosis Hospital. Sputum from the owner was also collected and typed at the same hospital. All contained the bovine type of tubercle bacilli. It was concluded, therefore, that the owner contracted tuberculous infection from the cattle which were slaughtered during or before 1929, but his disease did not become contagious until 1942. In the interim none of his cattle became infected.

In all likelihood, if this owner and his family had been periodically tested with tuberculin, he would have reacted in 1929. Annual examinations thereafter should have revealed his trouble prior to 1942, before it became contagious, when it could have been treated successfully with minimum inconvenience. By so doing he could have maintained not only his own good health but also that of his cattle.

Every person who has contagious tuberculosis should have the tubercle bacilli typed. The treatment and prognosis are not altered by the type found. However, if bovine bacilli are being disseminated by a person who is in contact with domestic animals and pets, particularly cattle, large financial losses may result as well as illness among other animals and human associates.

When typing of tubercle bacilli is done, generally some significant revelation may be in store. Through careful typing Horton found that 12 of his patients in the Homer Folks Sanatorium at Oneonta, N.Y., had pulmonary tuberculosis of the bovine type. Horton said:

> "The cases of pulmonary tuberculosis due to bovine type of tubercle bacilli which we have observed have been of about the same clinical pattern and have pursued about the same course as pulmonary tuberculosis due to the human type of tubercle bacillus. We cannot distinguish lesions caused by bovine bacilli from those caused by the human type in any other way than by bacteriological study. The lesions have varied in extent, distribution and intensity in the same way as human type tubercle bacillus infections do."

The avian, bovine, and human type of tubercle bacilli cause much of the disease in the species indicated by their names. Each one also causes significant and contagious disease in other species. For example, the avian type results in clinical disease in swine and sheep. Slightly more than 2 dozen cases in people have been reported. Moreover, the avian type is of great economic importance among fowls and swine. There is evidence that the avian type may cause nonprogressive tubercles which sensitize the tissues of various animals and humans so they react to tuberculin.

The bovine type of tubercle bacillus results in significant and contagious tuberculosis in numerous animals such as swine, horses, sheep, goats, cats, dogs, monkeys, and canaries. It is also a serious destroyer of human health and life.

The human type of tubercle bacillus causes progressive and contagious disease in such animals as swine, horses, dogs, monkeys, parrots, and canaries.

At one time it was thought necessary to have tuberculin prepared from different types of tubercle bacilli in order to test accurately for the disease produced by the various types in animals and man. For example, if one suspected that the bovine type of tubercle bacillus was responsible for the disease in the human body only tuberculin made from that type of tubercle bacilli should be used. It was found that this high degree of specificity does not obtain between the bovine and human types and that tuberculin made from the human type is entirely satisfactory in testing for both human and bovine infections.

Fowls and other animals including man infected with the avian type of tubercle bacillus may fail to react to tuberculin made from the mammalian types of bacilli, but react definitely to that made from the avian type.

Obstacles Encountered and Overcome

Never in history has so much been learned about tuberculosis with new knowledge so practically applied with such phenomenal results. However, every advanced step has been met by erroneous opinions and speculations of uninformed, misinformed, and inexperienced individuals. However, through their superior knowledge; clear vision of eradication; and firm determination to solve a large economic problem and to prevent people from becoming infected, falling ill, and dying from tuberculosis, they brushed aside all trivia upon which every obstacle was based and proceeded closer and closer to the eradication goal.

Although the number of obstructions which had to be overcome is legion, time permits only a few examples to emphasize their absurdity.

Opposition to the belief that the bovine type of tuberculosis is transmissible to people was rife and much was written and spoken in condemnation of the facts established by veterinarians and others. The following is from the Breeder's Gazette, 1901:

> The katabasis has come. For years the noble army of tuberculine squirt-gun manipulators has been marching up the hill, beating tom-toms and brandishing the pole-axe, crying, "Kill, Kill." This fierce and bloodthirsty campaign against our herds has been waged on the disputed assumption that tuberculosis in cattle is a menace to the public health. Broadly and yet accurately speaking it has been based on the discoveries and the declarations of Dr. Robert Koch, the eminent German bacteriologist, who at first believed he had found in tuberculin a cure for consumption. Servile worshippers of asserted authority, the half-baked scientists and zealots of the squirt-gun brigade have

pushed their work of destruction until it has mounted to millions of dollars, despite the protests of scientists of high standing who denied the alleged danger. This was the anabasis--the going up against the husbandmen of the world whose herds furnish milk and meat for the sustenance of the people.

But the katabasis has come. The going down has begun. In London last week before the scientists of the world assembled at the British Congress on Tuberculosis Koch recanted. He had previously affirmed the transmissibility of tuberculosis to man from the meat and milk of animals. After a decade of investigation he now declares with unqualified positiveness that "human tuberculosis and bovine tuberculosis are radically different diseases." He declares that he has absolutely demonstrated that cattle cannot be infected with human tuberculosis and that he has satisfied himself that it is impossible for bovine tuberculosis to be transmitted to man. The danger of infection from milk he considered "so slight as to be unworthy of precautionary measures."

Now watch the noble army of matadors march down the hill. But not with its banners of death's-head and cross-bones flying in the breeze; squirt-guns and pole-axes will be carried at "reverse arms." The man who first furnished inspiration and weapon has spiked its guns and it will fall back in a rout.

It should be remembered that Koch's London declaration is a recantation, a reversal of view, a signal surrender to stubborn facts discovered in years of investigation. But there are many scientists who have long held this clear vision. They have been in the minority, it is true, because the crowd blindly follows constituted authority. The theory of the transmissibility of tuberculosis through milk and meat has been held as "not proved" by many and has been vehemently disputed by some, armed with the facts of human experience and scientific investigation. Now that the acknowledged great authority on tuberculosis has cast aside his first-held views and joined the minority, a New York physician is doubtless correct in saying that "the enlightened minority of the medical profession, which has maintained that tuberculosis cannot be contracted from animal substances, will speedily become a majority of the profession."

But even assuming that some extremists, with the bigotry that holds to a preconceived opinion in the face of new and convincing evidence, try to continue to scare against milk and meat, in what position do the matadors find themselves? Simply this: standing on disputed ground, with the most eminent authority on tuberculosis in the world squarely antagonistic to their theory, they wage murderous warfare on the herds of the husbandman. It is against this outrage

> *The Gazette* has persistently protested. It raises no objection to a study of this disease at public expense; it has stoutly opposed the experimentation on private property which has characterized the tuberculin crusade and has demanded that it cease until more light is shed on the subject.
>
> Now a great light has shined. Truly enough "the sanitary systems of the world have been shaken to the very roots. The word revolution but faintly expresses what the discovery will precipitate." The supposed menace to public health from tuberculous cattle is wholly eliminated by Dr. Koch....
>
> But the reign of the pole-axe in the hands of the public official is doomed.

The author does not mention Revenel's strong rebuttal of Koch's presentation during the London meeting.

In 1904 a firm of druggists in New York City sent owners of cattle a letter which contained the following:

> Tuberculosis in cattle has proved to be more destructive to these animals than all disease put together. Aside from this, tuberculosis in cattle is more or less dangerous to human beings, especially to children, due to the consumption of milk. All measures taken so far to suppress tuberculosis in cattle have proved to be of no success: the strictest veterinary policy and the most thorough sanitary institutions, supported by the use of tuberculin, have failed to check tuberculosis.
>
> The latest method of Professor V. Behring, whose name is world renowned, seems to have solved the problem as to the suppression of tuberculosis in cattle. Professor V. Behring has been working on this subject practically for quite a number of years and his wonderful results are based upon thorough experimental work. We therefore have to deal with facts and not with theories.
>
> The method itself is comparatively plain and consists of two inoculations, the second one following the first twelve weeks later. The inoculation is especially opportune in calves and young heifers not exceeding the age of six months. The two inoculations will immunize them against tuberculosis for their lives. In order to render older cattle immune, a thorough examination, followed by a tuberculin test, has to be made before inoculating.
>
> We furnish the vaccine and have it also injected by our veterinarians, in order to be sure that it will be done properly.

Concerning this Leonard Pearson, Dean, School of Veterinary Medicine, University of Pennsylvania, said:

"It is not necessary for us to rely for our information upon the unsubstantiated claims of a business firm endeavoring to make money by the sale of vaccine against tuberculosis. Briefly, I may say, that the claims in relation to the work of von Behring are exaggerated and in part they are without foundation. There is absolutely not the slightest evidence to show that by means of von Behring's immunization method cattle may be immunized for their lives' or, indeed, that they may be immunized against tuberculosis from natural sources for a length of time sufficient to make the vaccinations at all valuable."

On March 17, 1905, the firm of druggists replied to Pearson's "unfounded charges." They had expected some opposition to the introduction of von Behring's bovovaccine, for they knew that all the great discoveries bring forth opposition and protests from inferior minds. In closing, the company registered a protest to Pearson's statement to the effect that its claims were of the patent medicine variety. Pearson won, as von Behring's vaccine proved to be a dud like all "vaccines" for tuberculosis before and since.

In 1909 the Reference Handbook of the Medical Sciences carried the following statement: "No effective control of bovine tuberculosis is possible. To use the tuberculin test for its detection and to destroy all cattle which give the reaction would result in economic losses impossible for the state to meet."

Although I have apologized many times both verbally and in print at least one more apology is in order for my following statement approximately one third of a century ago.

"The medical profession must bear in mind that cattle are capable of presenting evidence of tuberculosis infection in the absence of tuberculous disease. This fact should make us very cautious about supporting bills for legislative action that may carry an element of unfairness to the cattle breeders, the dairyman and even the general farmer. With our present knowledge we know how absurd it would be if laws were enacted compelling every person found to have tuberculous infection with no evidence of disease to take a long course of treatment. It is not equally absurd to compel the owners of cattle to have all animals slaughtered that show evidence only of tuberculous infection and accept the prevailing beef price for such animals? Laws making such action compulsory have sent many a man with the earnings of a lifetime invested in a thoroughbred herd into bankruptcy. Would it not be far better to enact or modify laws so as to make testing for infection compulsory and leave it to the veterinarian to determine which animals are actually diseased and a menace to others of their kind as well as to people who come in contact with them directly or indirectly? It has been fairly definitely proved that even by slaughtering all animals showing

evidence of tuberculous infection tuberculosis cannot be eradicated from the cattle family. It would seem, therefore, that our best procedure is to slaughter all animals suffering from tuberculous disease and to everlastingly teach the public the importance of pasteurization."

This is a clear-cut example of ignorance. It represents one of the most unfortunate practices among physicians--namely free and extensive expression of opinions without accurate information. Another good example follows.

Despite the well-established fact that the bovine type of tubercle bacillus is highly pathogenic for human tissues, we are still confronted with the occasional person who contends that a great injustice has been done man wherever tuberculosis has been controlled among cattle. An article published by Rainey in 1945 is capable of markedly retarding the completion of the eradication program should it be widely circulated among uninformed persons. He says: "Finally, the writer would summarize or define his thesis as, in effect, a plea for the abandonment of the test-and-slaughter policy for the control of tuberculosis or any other common disease of mammals on the grounds that it is in conflict with the nature of things, and consequently must fail in the end."

It was even taught that the bovine type of tubercle bacilli would produce mild lesions in the human body and immunize against later destructive disease. Therefore, it was argued that the bovine type of tubercle bacilli should be allowed to exist in dairy products in order to immunize children. Concerning this Schroeder (1927) countered:

> "With the indisputable knowledge that bovine tubercle bacilli cause tuberculosis in children, which, like tuberculosis in human beings caused by human tubercle bacilli, ranges from mild to severe and from severe to fatal, a knowledge which tuberculosis investigators who have studied the different types of the tubercle bacillus responsible for tuberculous disease in human bodies no longer question, it would be barbarous to permit, or to be indifferent to, the unguarded or uncontrolled exposure of helpless children to tubercle bacilli in dairy products..." "Exposure of this kind meaning, as it does, the indiscriminate ingestion of tubercle bacilli in doses, oftener than not, too large, too numerous, too virulent and untimely for safety, would be unpardonable, as it would have as one of its evil consequences the sacrifice of the health and lives of many children."

Lochead (1928) said: "A scientific theorist recently gave utterance to the thought that possibly milk from tuberculous cows tended to immunize children against human tuberculosis and we must expect some pseudo-scientist to urge us to cease our efforts to prevent tubercle bacilli occuring in milk. If it were true, which is unlikely, because no scientist has yet succeeded in immunizing against tuberculosis by the use of living tubercle bacilli, the method is haphazard, irregular and unscientific."

Despite all of the evidence to the contrary Holm (1946) wrote: "As long as tuberculosis among cattle prevailed in Denmark, the tubercle bacilli present in milk effectuated a vaccination against tuberculosis in the inhabitants, even though this was not intended." He apparently thought that all children should harbor tubercle bacilli and recommended BCG as a substitute. This hardly squares with Holm's statement that in parts of Denmark where tuberculosis was prevalent among cattle nearly half of the cases of pulmonary tuberculosis among farmers was produced by the bovine type of tubercle bacillus. He also reported that among 18,231 cases of pulmonary tuberculosis the bovine type of bacillus was responsible in 14.1 percent, and that among 4,186 cases of extra-pulmonary tuberculosis the bovine type was recovered from 19.8 percent.

Well-informed veterinarians were not deterred from their efforts, but such opposition did impede their work. They knew that most attacks were made by individuals who were uninformed or misinformed, and some were emotional even to the extent of being fanatical, some had political axes to grind, some had financial interests at stake, and so forth and so forth. For example, in 1902 Burneson, Ohio, said: "Tis true, many articles have been published by certain papers derogatory to the tuberculin test, but they have been written by those who never tested a cow, or perhaps never saw one tested. They have picked the dross from the gold and with it forged a sword, the wielding of which is only too apparent. This has been done for selfish motives alone, entirely regardless of the true conditions of the herds and the future outcome."

In 1921, Olin, Commissioner of Health of the State of Michigan, said that in a recent meeting of Michigan business men it was stated that "There is as much chance of getting tuberculosis from cows as getting hog cholera from ham and eggs."

The opposition was successful occasionally in having bills enacted into law which handicapped the program, and strong organizations were effected which temporarily stopped progress. In some places court rulings became necessary.

In 1905, Dean Leonard Pearson said, "There is scarcely a subject related to agriculture or public health that occasioned as much or bitter discussion, or has led to the expression of so many divergent views as this one of tuberculosis of cattle."

Solution of Present Problems

Inasmuch as the pathogenic types of tubercle bacilli produce disease in several species, including man, it is evident that these organisms must all be attacked with the goal of eradication if people are ever to be free from tuberculosis. Of all communicable diseases tuberculosis still heads the list in causing incapacity and death among the two billion seven hundred million people of the world. The bovine type of tubercle bacillus plays a significant role in this monstrous public health problem, which can be solved only when veterinarians and physicians in human medicines join hands and together with their allies,

and indeed the entire citizenry, make an all-out attack on the pathogenic types of tubercle bacilli until the last one has been destroyed.

The veterinary profession has demonstrated the method by which this disease can be eradicated from animals so overwhelmingly that their accomplishments have the designation of "Man's Greatest Victory Over Tuberculosis." For a third of a century physicians in human medicine have known how to solve the problem equally well in people. In fact 25 years ago an editorial appeared in a medical journal under the title, "What are We Waiting For?" In tuberculosis work among humans we are still waiting because no person or organization has had the vision or the courage to attack tuberculosis on a nationwide basis in a manner consistent with the magnitude of the problem it presents.

The value of the Tuberculosis Christmas Seal has been immense, but the money derived from it is a mere pittance compared with what is necessary to eradicate the disease.

In people it continues to cost an amount approaching close to a trillion (a million million) dollars annually. In 1917, veterinarians and their allies launched a program consisting of periodically determining the status of every bovine in the Nation and acting accordingly. In the past 40 years the total cost was approximately $326 million. However, this work resulted in a saving now being enjoyed by cattle owners of approximately $150 million annually. This well-nigh miraculous accomplishment was achieved by establishing facts by basic research and proceeding with applied research.

For more than a quarter of century before the eradication goal was set, the finest kind of fundamental and practical research resulted in establishment of facts which revealed the natural history of tuberculosis in the animal body. All fundamental information was transmitted to students and graduate veterinarians everywhere.

In this country the findings of Gutmann, Bang, and others with reference to specificity of the tuberculin test were first confirmed by Pearson and Cotton, in Pennsylvaina in 1892, and Russell, in Wisconsin in 1894. Following these early demonstrations, this test was widely used by veterinarians throughout the United States with overwhelming proof of its diagnostic value. At post mortem, areas of disease were found with such frequency in tuberculin reactors that a reaction became synonymous with the presence of lesions. However, just as Bang had observed, in the occasional reactor no lesion was found. In 1923, Calmette said: "Tuberculin has frequently been accused of having a false indication because no tuberculous lesions could be found....It has been proved, however, long ago that in these circumstances the organs had not been searched with sufficient care. Whenever a tuberculin reaction is positive, there exists somewhere a follicular lesion or at least a gland containing tubercle bacilli whose presence can be disclosed by experimental inoculation of the guinea pig." Schroeder in 1924 cited a number of instances in which tuberculin reactions among cattle were not explained until long and tedious post-mortem examinations not possible at slaughterhouses revealed tuberculous lesions in unusual and unexpected locations and in organs rarely involved.

As time passed many well-informed veterinarians and physicians in tuberculosis work among humans believed that "no lesion tuberculous reactor" was not a true statement; therefore, the phrase "no visible lesions" was substituted, and now "no gross lesion found" is in vogue. These veterinarians and physicians agreed with such persons as Calmette and Schroeder. Their contention was supported in the past by Nassal (1956), who did more extensive necropsis on 1,000 bovine carcasses in which no lesion was found on routine post-mortem inspection. In this manner lesions were found in 50.2 percent of this so-called no-gross-lesion-found group. It is possible that still more meticulous necropsis such as Ghon did on 184 children might bring to light a larger number.

Original observations were so completely confirmed that informed veterinarians everywhere accepted the tuberculin reaction as proof of the presence of tubercle bacilli, regardless of minuteness of the lesions containing them. Thus, the profession accepted the tuberculin test at its sole diagnostic agent prior to the appearance of extensive disease and the presence of tubercle bacilli in secreta and excreta. Hence, veterinarians were able to diagnose tuberculosis within approximately a month after the animal's body was initially invaded with tubercle bacilli, while the lesions were still microscopic and before the disease was contagious and for as long thereafter as tubercule bacilli remained alive in the animal's body.

Observation revealed that healthy appearing animals which reacted to the tuberculin test might at the moment or at any subsequent time have contagious lesions. Therefore, every tuberculin reactor was looked upon as a reservoir of tubercle bacilli then or potentially capable of being disseminated among their associates. Inasmuch as no germicidal drug was available and no effective treatment was known, common sense dictated that all tubercle bacilli so found be destroyed with the animals.

So much research work was done and the eradication method was proved so efficacious that it was administered in a nationwide way beginning in 1917. Between then and now, 404,874,447 tuberculin tests have been administered to the cattle of this country and routine post-mortem inspections have been made on the carcasses of 4,096,909 reactors. This is the largest, well-organized project ever conducted anywhere at any time. The book entitled "The Conquest of Bovine Tuberculosis in the United States" by H. R. Smith, Somerset, Mich., should be made available not only to all members of 4-H Clubs and Future Farmers of America, to whom it is dedicated, but to citizens everywhere, as it is an accurate description of the tuberculosis-eradication-among-animals campaign leading to the most phenomenal results of all time.

It appears that those who have accomplished so much for public health are never freed from harassment. After contending with strong opposition to every effort to eradicate tuberculosis from animals and defeating each opponent in turn on the way toward victory, one would expect that the entire citizenry and especially those informed in any aspect of public health would take unlimited pride in the accomplishment to date and would cooperate to the nth degree in traveling to the eradication goal.

Apparently the number of workers in most public health projects willing to carry through to complete accomplishment is somewhat limited. When the glamour is gone and the drudgery of the final cleanup begins, many lose interest and their activities cease. This leaves the public with the impression that the problem has been completely or nearly solved, and a general spirit of complacency prevails. Dr. Anderson, Director of the Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, said (1959):

> "Complacency may be defined as contentment and satisfaction--especially self-satisfaction--which, in this case, resulted in the destruction of enthusiasm, initiative, and inspiration so essential to successfully carry out the eradication effort.
>
> "Against the strenuous efforts of some, complacency progressively and effectively penetrated the program during the 1940's. Tuberculosis eradication was displaced in public, industrial, and professional circles from a position of pre-eminent concern to one of second-rate importance, due, in major part perhaps, to the great publicity given to so-called "Achievement Days" celebration when states were first designated as modified-accredited areas. While the status of modified accreditation was considered an essential milestone in reaching the objective of eradication, the importance attributed to having reached this status naturally led to a letdown. All of this stimulated popular acceptance of false concepts, which were that: (1) control measures were adequate; (2) an irreducible minimum had been reached in tuberculosis eradication so that industry would have to live with a certain amount of bovine tuberculosis; (3) tuberculosis was a vanishing disease and no longer an economic problem; (4) victory had been won, and to continue pushing the eradication effort was a waste of time and money; (5) bovine tuberculosis as a public health hazard was non-existent; and (6) the need for vigilance was gone and the pressures for vigorous action withdrawn."

One might anticipate manifestation of complacency when such phenomenal accomplishments have been achieved as those in bovine tuberculosis with only 0.231 percent infected among those tested in 1959 and the losses from condemnation at the points of Federal inspection being at such low ebb. However, in view of the complacency now being manifested by the citizenry, the many tuberculosis organizations, medical associations, and so on, and so on, with reference to the eradication of tuberculosis among people are an enigma. There is nevertheless, evidence to indicate that tubercle bacilli have taken refuge in the bodies of more than 50 million people of the United States, of whom 60,000 or more become clinical cases and approximately 12,000 die each year. Although the number is far smaller than a few decades ago, it is still staggering to learn that a new case is reported every 6 minutes and one dies every 45 minutes.

Complacency concerning tuberculosis in people does not differ much from that among animals. Among people the mortality rate has been falling

for many years primarily because of the work of sanatoriums, protection against bovine type of tubercle bacilli, and recognition of the two specific diagnostic agents. The number of clinical cases was decreasing correspondingly. However, when it was announced that "miracle" drugs were here and the disease could be "cured" by drugs and resectional surgery, many workers, including physicians, all too often predicted the end of the tuberculosis problem in a few years. Such unfortunate predictions caused much concern among full-time tuberculosis-organization workers. With tuberculosis departing so soon some arrangement must be made for their future; therefore those who did not analyze the situation or seek more mature advice began to advocate that funds provided for tuberculosis be used to extend their activities to other diseases. Names of some tuberculosis organizations and their official publications were changed, with mention of tuberculosis in a minor position or absent.

Because of the decreased number of sanatorium admissions and the unfortunate prediction that the job of these institutions was done, a sizeable number of sanatoriums were closed and their professional staffs dispersed. No staff member was left--and there was no plan for expert periodic examination of the former sanatorium patients, many of whom are certain to relapse; no arrangement was made for expert management of those still on antituberculosis drugs. No one was there to locate in the respective areas served, all the persons in whom tubercule bacilli had taken refuge and to keep these persons under close surveillance.

Some physicians became almost fanatical concerning the value of the recently acquired drugs, stating that no sanatorium should be permitted to exist unless it had a laboratory equipped to study resistance of tubercle bacilli to them and a first-class department of surgery. Some sanatoriums were closed for lack of these facilities, despite the fact that they had long been affiliated with excellent laboratories in other institutions as well as first-class surgical facilities. Some sanatoriums were converted into institutions for more popular activities such as care for the aged.

Every time a sanatorium was closed complacency reached a high degree in the public mind. Obviously, in all such places tubercle bacilli had been unleashed, and there was little likelihood of any effort to be made to corral them before their destructiveness reaches an extreme degree as manifested by illness and death. This may require from a quarter to a half century, but when it does occur it will be the result of unjustified complacency.

Apparently lack of information and complacency caused one physician to write (1959): "Today especially in a state like Minnesota and in a good many states the value of routine tuberculin testing of school children can be seriously questioned." "Certainly it would appear sound to use the funds and personnel time to more productive activities at present." Such expression based almost entirely on personal opinion impedes progress in the eradication program. This person's experience with tuberculosis has been too limited to justify such statements. Moreover, they were made in a State where the tuberculosis program has been most carefully considered by students of the disease. There the

State Board of Health, the State Tuberculosis Association, and the State Medical Association have recommended without reservation certification of schools so that at least 95 percent of the children and 100 percent of the personnel will be tested with tuberculin with appropriate followup procedures.

To overcome complacency and reestablish interest and activity in tuberculosis is a much more difficult task than it was to establish programs 40 to 50 years ago. Then the disease was in the limelight, cattle were consumptive and died, numerous families had sick members and many died. The problem was easily visualized, and efforts to solve it were supported. Now, little is to be seen and "out of sight, out of mind," is applicable.

However, it should be possible to retrieve a sufficient number of workers to rejoin those who are still laboring with the hope of dispersing fallacies and replacing them with facts in the minds of our citizenry. The 1958 conference and this one will be extremely helpful and should set the example for all organizations to return to their jobs and solve the most serious of all communicable-disease problems. What must be done has been known so long and has been said so many times, but continued repetition is necessary:

1. A program must be developed in which all professional workers participate. It has even been suggested from several areas that in order to accomplish tuberculosis eradication an organization limited to this objective be established, composed of veterinarians, physicians in human medicine, nurses, social workers and all other dedicated persons engaged in every aspect of health work, and also the entire citizenry. The objective would be, not just to stop death and illness, but to destroy the last tubercle bacillus.

2. It must be made clear that all necessary basic research in tuberculosis has been done and has produced sufficient armamentarium to eradicate the disease. Workers in the field of cancer are where we were prior to 1882. Research must be their watchword to determine etiology, produce a specific diagnostic test, adequate treatment procedure, and possibly preventive measures. Because basic research is so necessary and is so emphasized in cancer and certain other diseases, there seems to have developed an impression that no tuberculosis program is complete without major activites in basic research.

3. An all-out effort must consist of locating those persons and animals who harbor tubercle bacilli. As long as such organisms remain undiscovered there is little to be done to prevent them from wreaking havoc in the bodies of their hosts and spreading to other people and animals. Nothing but the tuberculin test will locate both the human and animal hosts of tubercle bacilli. The test is simple to administer, harmless, inexpensive, and yet the most accurate test available for any disease.

4. It must be possible to remove and destroy animals found to react. People who react must be examined promptly. If lesions have not evolved

to macroscopic size so as to be located by present methods of examination they should be examined periodically thereafter in order to detect such lesions promptly if and when they evolve.

5. All persons found to have clinical disease on initial or subsequent examination must be treated promptly. If already contagious they should be isolated at once and treatment continued until they are able to resume activities of life without endangering their associates.

6. People everywhere should be informed that dependable immunity never develops in tuberculosis. Therefore, there is no premise for devoting time, effort, and funds to produce immunity artificially. It is important that people everywhere be informed that all hope of eradicating tubercle bacilli by so-called immunizing agents is forlorn.

7. No board of health, local or State, should operate without one or more veterinarians included in its membership. To date this obtains in only a few places. No tuberculosis association, National, State, or local, should operate without including in its membership every interested veterinarian in the area it serves. Veterinarians should occupy key positions and hold various offices of the organization. In only a few places has this been the case.

8. The seriousness of the present tuberculosis problems must be emphasized and reemphasized at every opportunity. Members of professional groups should have articles appearing regularly in their official journals calling attention to the seriousness of the problem and stating the facts concerning the necessary steps to solve it. Such information should also be heard on radio programs, seen on television screens, and read in popular magazines and newspapers everywhere.

TUBERCULOSIS CONTROL IN HUMANS

Lewis S. Jordan, M.D., Superintendent, Riverside Sanatorium
Granite Falls, Minn.

During 1929 and 1930 I was priviledged to work with Dr. C. E. Cotton, D.V.M., in accrediting the last two counties in Minnesota in bovine tuberculosis control. The counties were accredited.

Inspired by the accomplishments of the work of the veterinarians in the eradication of tuberculosis among the cattle by the use of the tuberculin test, it was decided in 1930 to institute a similar, but modified, program in the Riverside Sanatorium district of Minnesota. It was felt at that time that intensive Mantoux testing of the school children and young adults with sufficient follow-up work would lead to the various open cases, which were the infective factors in the areas. With this in mind, plans were formulated.

This district, with an area of 3,800 square miles, is located in the south-central and western part of the State and comprises four large counties, Chippewa, Yellow Medicine, Lac qui Parle, and Renville. It has 27 small cities and towns and approximately 265 rural schools. The tuberculosis control work as planned in 1930 had the following aims:

1. To discover the infected children so they could be observed throughout their school career and on into adulthood.

2. To trace wherever possible the source of infection for each child found to be a tuberculin reactor.

3. To eliminate the infective factor by breaking the contact with the child, preferably by institutionalizing the persons with positive sputum, or, otherwise controlling their disease.

4. To examine all adult contacts of the children, in the school, including teachers, janitors, bus drivers, cooks, office help.

5. To institute an educational program including talks, movies, and literature to reach all lay groups and civic organizations.

6. To institute a follow-up program, checking each tuberculin reactor by roentgen ray films of the chest, at least once a year, or more often if indicated.

7. To obtain 100 percent cooperation of the State and local medical societies.

8. To aid the veterinarians' program of eradication of tuberculosis among animals, particularly cattle so no human would be infected with the bovine type of tubercle bacilli.

With this eight point program as a guide, work was started. An intensive educational program was begun to gain the cooperation of school boards, lay organizations such as Rotary, Kiwanis, Lions, commercial clubs, American Legion, parent-teacher organizations, farm groups and organizations.

With the backing of these organizations, and a ruling by the Attorney General, Harry H. Peterson, "that the Mantoux skin test for teachers and other school employees is a reasonable exercise of police powers of the school boards, if required for the purpose of safeguarding the health of the pupils of public schools," the Mantoux surveys were instituted.

School boards could legally require all employees, including teachers, to prove themselves safe to act as guardians of children during the school hours. Each child was given a blank form, to be signed by the parent or guardian, giving consent for the administration of the Mantoux test and, if a reaction occurred, a roentgen ray film of the chest. No test was done without written permission. At the same time it

was explained that it would be necessary to roentgen-ray all reactors, and, if the individual were able to pay, a fee $1 would be charged to partially cover the cost of the film. The additional cost of the entire examination was derived from Christmas Seal plus Sanatorium money. For persons unable to pay, the entire expense was borne by this fund. All reactors were roentgen-rayed regardless of their ability to pay. Care was taken to explain that the $1 did not cover the cost of the work and materials and that the balance was provided by Christmas Seal money and the Sanatorium.

The goal was high even in the early days of the program. The rule was established that the children of no school would be tested unless consent blanks were returned by a minimum of 80 percent of the pupils. This placed the responsibility of the testing program on each community where the work was to be done. Parent-teacher groups worked hard to obtain the 80 percent consents. A spirit of rivalry developed between various towns and schools, which practically always assured the success of the program. Every effort was made to retest the schools each 2 years. Saranac Lake old tuberculin 1:1,000 strength was used in the tests. Comparison of O.T. 1:1,000 and PPD were used on certain occasions during the years, as were readings at 48-to 72-and 96-hour intervals. As a result of the latter observation, it was decided that a 72-hour reading is, for general purposes, in all probability somewhat more accurate than the 48-hour intervals owing to the fact that it detects some of the so-called "delayed reactors," as well as eliminates the possibility of interpreting small areas of erythema due to trauma and such as tuberculin reactions.

In every instance diligent search was made to determine where the reactors received the infection. In this the field nurse and the knowledge of the family physicians proved of great value. In many instances, the source of infection was found in a grandparent, an uncle, an aunt, hired man, who had unsuspected or undiagnosed infectious tuberculosis. During the first several years that this program was conducted in schools, 12 teachers were found with demonstrable pulmonary tuberculosis of which 8 had positive sputum. In one room, in one of the larger schools, 42.6 percent of the pupils reacted to the Mantoux test. The teacher also reacted and the roentgen ray film of the chest revealed evidence of disease which was proved to represent far advanced "C" pulmonary tuberculosis. This teacher died in a sanatorium within 60 days of discovery. Of the pupils who reacted to tuberculin in his room, two developed demonstrable active pulmonary tuberculosis within a year.

All teachers and school employees found to be a menace insofar as tuberculosis infection was concerned were removed from their contact with the children. This work went on yearly with pleasing results. More than 150,000 children were tested, and the percentage of tuberculin reactors found in the same schools decreased as the years passed.

The following tabulations show the decrease in the percentage of tuberculin reactors observed during the first 20 (or 30) years of the surveys:

MANTOUX SURVEYS, 20-YEAR COMPARISON OF SCHOOLS

	Year	% Tested	% Reactors
Olivia	1934	88	11.5
	1953	99.7	5.1
Buffalo Lake	1932	73	12.8
	1953	99.4	7.2
Clarkfield	1933	47	9.6
	1952	100	3.4
Granite Falls	1931	74	9.4
	1953	95	4.7
Wood Lake	1930	81.4	7.9
	1952	98.4	2.3
Bird Island Public	1932	81.7	25.7
	1952	95	3.8
Bird Island Parochial	1934	47	19.4
	1953	94	4.2
Sacred Heart	1931	66	26.2
	1952	100	3.3
Franklin	1934	89	12.2
	1953	95	3.8
Boyd	1931	85	15.6
	1952	96	1.1
Fairfax	1934	67	21.3
	1953	95	4.2
Marietta	1931	54	17.9
	1953	97.2	2.8
Hanley Falls	1933	75	11.7
	1953	100	3.2
Milan	1933	80	13.3
	1953	95	3.4
Madison	1933	74	14.1
	1952	98	5.8
Montevideo	1933	80	13.3
	1952	93	3.4
Morton	1933	86	13.6
	1952	97	6.1

MANTOUX SURVEYS, 10-YEAR COMPARISONS OF ALL SCHOOLS, BY COUNTY AND GRADE

RENVILLE COUNTY

Period:	Grades inclusive	% Reactors	Number tested
1930-1939	1st to 6th	9.01	
	7th to 12th	18.2	
1940-1949	1st to 6th	2.5	
	7th to 12th	5.8	11,448
1950-1958	1st to 6th	2.4	
	7th to 12th	6.2	24,493
1950-1958	Percentage of total enrollment tested	96.0	

LAC QUI PARLE COUNTY

1930-1939	1st to 6th	7.3	
	7th to 12th	10.9	
1940-1949	1st to 6th	2.2	
	7th to 12th	6.0	6,413
1950-1958	1st to 6th	1.9	
	7th to 12th	4.6	11,720
1950-1958	Percentage of total enrollment tested	94.5	

CHIPPEWA COUNTY

1930-1939	1st to 6th	5.4	
	7th to 12th	7.3	
1940-1949	1st to 6th	2.1	
	7th to 12th	5.2	6,290
1950-1958	1st to 6th	2.5	
	7th to 12th	5.03	13,612
1950-1958	Percentage of total enrollment tested	97.7	

YELLOW MEDICINE COUNTY

1930-1939	1st to 6th	5.4	
	7th to 12th	7.3	
1940-1949	1st to 6th	2.1	
	7th to 12th	5.2	8,509
1950-1958	1st to 6th	1.47	
	7th to 12th	4.9	14,832
1950-1958	Percentage of total enrollment tested	98.8	

When this work was begun in 1930, an average of 13.9 percent of all of the school children reacted to tuberculin.

Following a report, the Minnesota Sub-Committee of Tuberculosis of the American School Health Association (consisting of such persons as Dr. E. A. Meyerding, St. Paul; Dr. J. A. Myers, Minneapolis; Dr. L. S. Jordan, Granite Falls, and Dr. S. A. Slater, Worthington) was organized. In 1943 this committee prepared standards by which individual schools or whole systems in Minnesota might be certified with reference to tuberculosis. In order for a school to be certified, it must meet these standards which pertained to tuberculosis control work in progress. Those meeting the highest standard were offered Class "A" certificates, while those with slightly lower qualifications would receive Class "B" certificates. The requirements for Class "A" certification are, briefly, as follows:

1. Testing from 95 to 100 percent of pupils and making roentgen-ray films of the chest of all reactors.

2. Testing 100 percent of the school personnel and requiring roentgen-ray films of the chest of all reactors. This included teachers, janitors, bus drivers, cooks, clerical help.

3. Completing the examination of all who presented roentgen-ray shadows which might be caused by a tuberculosis lesion.

4. Conducting an educational program for the staff of each school so that the principles of tuberculosis control would be understood.

5. All nonreactors to tuberculin to be retested each 2 years.

6. All reactors to be roentgen-x-rayed each year, unless more frequent inspections were especially indicated.

For the Class "B" certification the requirements were exactly the same as for Class "A" except that only 85 to 95 percent of the pupils had to be tested.

In January 1946, work was begun in an attempt to accredit as many schools as possible under this new program of the American School Health Association. Work was started to accredit all schools in our Sanatorium District. It progressed very well. As an example, during our 1958 program 216 schools were accredited with 179 "A" certificates and 37 "B" certificates. One hundred and fifty-one schools were 100 percent tested. In 128 of these there were no reactors. Of the total number of schools tested, 152 schools had no reactors. There are now 3,552 certified schools in Minnesota.

We believe, from experience, that most of the cases of pulmonary tuberculosis in the years to come will develop among the group of children who are reactors to tuberculin today. Therefore, we believe that not

only control, but also eradication, of tuberculosis can be accomplished by the foregoing program. It is a matter of starting at the "grass-roots" to eliminate tuberculosis. In otherwords, begin with the child to find the infection, then discover its source. Eliminate the fountain-head of tubercle bacilli, either by controlling the disease or by hospitalization. Carefully watch the infected children; make roentgen-ray film inspection of their chests annually as they approach and continue on into adulthood. If roentgen-ray shadows appear, complete the examination for tuberculosis. A diagnosis is not made on a roentgen ray alone.

The weapons are now in our hands. They must be used with arduous work to eliminate tuberculosis. The main weapons are the Mantoux test, the roentgen ray, the field nurse, and the family physician. No one of them is adequate. They must be used together to conquer tuberculosis. In areas where there is a low incidence of tuberculosis, we feel that the countywide tuberculin testing program should always come first; the roentgen-ray inspection of the chest then can be limited to the reactors rather than a roentgen-ray mass program.

When a tuberculin reaction is present, two important facts are immediately established: (1) The individual is at least a potential case of clinical tuberculosis, (2) There has been a source of infection which may be sought and often is found among the individual's adult contacts in daily life.

The simple fact that a person reacts to tuberculin immediately arouses in him or her an intense interest in tuberculosis, and he or she desires information concerning the ultimate outcome of this infection. The logic of periodic examinations including roentgen-ray film inspection of the chest comes to be obvious to them, and their cooperation is easily gained. On the other hand, if only roentgen-ray film inspection is made, as practiced in mass roentgen-ray surveys, and the findings are reported as normal, it is often exceedingly difficult to maintain that individual's interest in subsequent periodic examinations, which often may be indicated. A tuberculin reaction gives a definite hold on the individual and affords the knowledge that they have been infected. This knowledge alone in most cases assures one of full cooperation thereafter. It seems far more important to concentrate attention on the tuberculin reactors, in whom potential clinical tuberculosis exists, than to devote time, energy, and money to making roentgen-ray inspections of the tremendously high number in whom there is no possibility of finding clinical tuberculosis. It is believed that tuberculosis in rural Minnesota can be eradicated by this method.

I feel that it would be a tragedy to introduce BCG in an area such as ours. To artifically establish sensitivity of the tissues of all of the citizens would be to destroy our most potent weapon--the tuberculin test. It must be remembered that in order to develop clinical tuberculosis, infection must first occur. If one eliminates the sources of infection and carefully watches those few whose tuberculin tests shows that they have been infected, there is every reason to believe that tuberculosis will be relegated to same category as typhoid fever and smallpox in the entire State within the life span of the coming generation.

TUBERCULOSIS--TOTAL ERADICATION

Ralph L. West, Secretary and Executive Officer,
Minnesota Live Stock Sanitary Board

For nearly 40 years, veterinarians and other persons interested in livestock sanitation have been talking and thinking about total eradication of bovine tuberculosis from the United States. When the campaign to accomplish this purpose by testing all cattle under the area plan was initiated, this disease was widespread and firmly entrenched throughout the Nation. In spite of skepticism on the part of many well informed livestock sanitary authorities, and in spite of strenuous opposition, both organized and by individuals, phenomenal progress was made, resulting in the declaration in 1940 of all counties in the United States as Modified Accredited.

Unfortunately, the declaration at that time designated such counties as "Modified Accredited Tuberculosis-Free." This was unfortunate terminology, and perhaps contributed to the complacency with respect to tuberculosis which has become widespread in recent years. Whatever the reason, there has developed a feeling of accomplishment, not only by the livestock industry but by many veterinarians, which is not justified while centers of infection still exist, widely scattered as they may be.

It is true that economic losses from tuberculosis are now insignificant when compared to those suffered before the eradication program was initiated, and human cases of bovine origin, particularly once so prevalent in children, have practically disappeared. The fact remains, however, that the program not only has not been brought to its final conclusion of total eradication, but the remaining centers of infection constitute a constant threat, capable, if ignored, of undoing in a few years the tremendous job already accomplished at great expense and effort.

During the last 20 years, little progress has been made toward wiping out the final vestiges of tuberculosis. This has necessitated constant vigilance and repeated testing to maintain the present status at a heavy expenditure of public funds. It seems clear that our procedure needs reevaluation and that a further program needs to be devised to attain the end we have been striving for--total eradication. For some time, we have been considering the possibility or practicability of instituting a revised area plan devised to complete eradication of tuberculosis, not only from cattle but from all species of domestic animal and domestic fowl. Because of the close association of different animal species and the possibility of interspecies infection, or at least sensitization, such a program appears to be the only answer with our present knowledge.

To be successful, such a program must, of course, include all animals within an area and must include provisions to protect livestock from exposure to human beings who may be spreading the disease.

Obviously, such a program presents staggering obstacles. However, the only alternative seems to be the continuation of systematic testing of all cattle for an indefinite time.

Among the problems that must be solved before such a program can be initiated with any hope of accomplishment are the following:

1. Public Apathy. Economic losses from this disease have been small in recent years. The cost of continued testing is borne by the State and Federal Government, and the individual taxpayer is not aware of the constant drain on public funds. Some means must be provided to reawaken public interest in complete eradication of this disease. Public support for such a program is essential. First, because of the necessity for full participation on the part of livestock owners; and second, because the funds required for success of such a program can only be obtained when there is a public demand therefor. The Animal Disease Eradication Division of the Agricultural Research Service is to be congratulated on their efforts toward this end by holding meetings such as this and other types of public information.

2. Opposition by Livestock Shippers and Dealers. Any successful plan for total eradication will require provisions to prevent reintroduction of diseased animals during the intensified eradication program and after the disease has been eliminated. Movement of healthy livestock is an accepted and necessary part of our agricultural economy. Great care must be exercised in adopting provisions that will not unduly interfere with this procedure, and will, at the same time, accomplish the purpose desired. Any program of this nature will most certainly be opposed by those interested in the movement of livestock. The opposition will be difficult to overcome unless there is real public support for the program.

3. Quarantine of Infected Premises. Strict maintenance of quarantines of premises which are found to be infected, or which will become infected from time to time in spite of all efforts, will involve continuous vigilance by trained personnel at a substantial cost until complete eradication has been accomplished. Paper quarantines without constant surveillance will certainly not be effective. Enforcement of such quarantines, again, is contingent on public support and will be impossible without it.

4. Prevention of Transmission of Tuberculosis to Livestock From Infected Human Beings. Some means must be provided to prevent contact of infected human beings with susceptible livestock. This will involve disposal of human waste on farms as well as from municipalities so that livestock cannot gain access thereto. Also, provisions must be made to eliminate affected humans as caretakers and handlers of livestock. Since such measures will involve, to some extent, freedom of choice of employment, this problem is perhaps the most difficult one to overcome.

5. Full Participation. Since, to be of any value, a project of this nature must have full participation by all livestock owners. Legislation will be necessary providing that all livestock shall be submitted to the tuberculin test when their owners are directed to do so by livestock sanitary authorities, and providing the requirement of proper disposal of all reacting animals. Also, since prompt disposal of all reacting animals will be essential, probably the payment of indemnity will have to be considered

The foregoing obstacles to an all--out tuberculosis eradication campaign have been mentioned only in the hope they may be avoided, and should not in any way be considered as deterrent to the adoption of a sound program. Unless we make continued progress, we can only hold our own by constant and systematic testing of all cattle, and the elimination of all reactors. Failure to maintain such a testing program will surely lead to retrogression, and there is plain evidence that such retrogression has occurred in some areas in recent years. If we are not to lose what we have already gained in this war against tuberculosis, we must in addition to devising a further program, which clearly will be slow of accomplishment, continue our present testing program to the fullest extent possible with available funds.

It is true, of course, that much knowledge concerning tuberculosis is yet to be gained through diligent research. Increased knowledge, as it becomes available, will surely make the task of eradication easier and more readily accomplished. On the other hand, it is impractical to delay plans for further progress toward eradication until more knowledge becomes available. Livestock sanitary agencies would have accomplished little indeed in the control and eradication of any disease, if programs devised for that purpose had awaited full knowledge of all aspects of the disease to be dealt with. In the case of tuberculosis, by using the weapons at hand tremendous progress has been made, and if we use all our present knowledge to the utmost of our capabilities, further progress can and will be made.

To summarize:

1. It is essential to devise a mopping up program to eliminate the last remains of bovine tuberculosis.

2. Such a program should include all species of domestic animals and protection of domestic animals from infected human beings.

3. While such a program is in progress of development, and during the long period before it can be universally adopted, we cannot afford in any manner to relax our efforts under the present programs, and these must not only be continued, but intensified.

V. BROADEN OUTLOOK THROUGH EPIDEMIOLOGY

PRINCIPLES OF EPIDEMIOLOGY

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I had the opportunity to read the proceedings of the 1958 Tuberculosis Eradication Conference and became impressed with the epidemiologic philosophy interwoven in most of the presentations. Also, I quickly realized that physicians of veterinary medicine have an unusual record in practicing epidemiologic principles in combating infectious diseases. In view of the outstanding contributions made in the field of epidemiology by veterinarians and the significant role of veterinary medicine in the control and prevention of some of the most damaging diseases affecting human populations as well as domestic animals, I wish to express some surprise that a physician of human medicine has been honored by having the privilege of addressing this group on the principles of epidemiology. The only reasonable explanation that comes to my mind is the astuteness of physicians of veterinary medicine to realize that the same epidemiological principles are used in all professional fields.

Epidemiology is a field of scientific endeavor which is still evolving and consequently is being refined and redefined as knowledge in the biological science increases. With new knowledge there is better understanding of biological processes which determine health and disease and greater appreciation of the epidemiological approach to medical and health problems.

Epidemiologic principles are equally applicable in studying the natural history of noninfectious disease as well as infectious diseases. This discussion will be limited however, to the presentation of epidemiologic principles of infectious diseases.

The basic question which epidemiologists are seeking to answer when investigating the occurrence of a disease or an outbreak of an epidemic is, "Why did this happen?" It is sometimes phrased as follows: "Why did <u>this</u> person (or animal) become ill and not <u>that</u> one?" or, "Why did <u>these</u> people (or this herd) become ill or infected and not the others in this locality?" If an answer can be found to the "Why" questions, then effective and practical control measures can usually be devised and applied. Thus epidemiology may be said to be the cornerstone of preventive medicine and public health.

In contrast, the practicing veterinarian who is called to see a sick animal is chiefly concerned about getting an answer to the question, "What illness does this animal have?" When the veterinarian finds the answer to this question he is able to prescribe the appropriate drugs and institute a proper regimen of treatment.

There is often a close relationship between the efforts of the practicing veterinarian and the epidemiologist. In many instances if the epidemiologist discovers what the illness is it is rather simple to answer the question why this illness appeared in the sick animal or why this outbreak occurred among this herd. The practicing veterinarian, likewise, may readily determine what the illness is if he used an epidemiological approach of finding out the circumstances connected with the appearance of this particular illness in the sick animal.

Finding the answer to the question of why a disease or an epidemic occurs is often difficult. This difficulty is due to the fact that an infectious disease represents a dynamic biological phenomenon which takes place between two very complicated living organisms--a micro-organism (the parasite) and a host (man or animal). In this relationship between host and parasite each is trying to survive and maintain its existence, if necessary at the expense of the other.

1. If the relationship between the two happens to be a very unsatisfactory one, either (a) the host becomes critically ill and may die, or (b) the parasite is quickly eliminated or may be destroyed.

2. If the relationship is a more satisfactory one, then both the host and parasite may survive, but the host may suffer some degree of illness manifested either as a typical clinical case or as an abortive attack.

3. If the relationship is more or less an ideal one then both the host and parasite survive with slight, if any, detectable damage to the host. This relationship is often referred to as an inapparent, latent, or subclinical infection. Special tests such as a positive tuberculin test may be the only indication of the presence of the parasite.

Both host and parasite are intricate and complex biologic entities and as such possess identifying biologic characteristics and attributes. For example, the host has such biologic characteristics as age, sex, race, state of nutrition, degree of immunity. The epidemiologic significance of these attributes are fully appreciated by veterinary medicine. Two classical examples in veterinary medicine illustrating the influence of age (the very young animal versus the mature animal) upon disease transmission and clinical manifestations are Texas tick fever and brucellosis.

The parasite may have such characteristics as motility, spore forming ability, exotoxin producing ability, host specificity, immunizing ability, specific nutrient requirements, and degree of stability. The host and parasite characteristics and attributes greatly influence the appearance, distribution, frequency, and character of the reaction (disease) between the host and parasite.

The mere presence of the host and parasite within the same environment will not necessarily initiate the biologic reaction we call

disease or infection. There must be an effectual combination of the host and parasite for disease to occur. For example, swallowing tetanus bacilli will not result in tetanus, nor will the contamination of a deep penetrating wound with pinworm eggs be expected to produce pinworn disease. The importance of effectual combination of host and parasite has been recognized for a long time and diseases are frequently classified epidemiologically on the basis of how the effectual combination is attained. For epidemiologic purposes diseases are usually classified as:

1. Respiratory-borne diseases.
2. Gastrointestinal-borne diseases.
3. Insect-borne diseases.
4. Contact diseases.

The environment through its influence on the host and parasite plays an essential role in disease occurrence and epidemic outbreaks. Environmental factors are often largely responsible for the conditions which bring the host and parasite together in an effectual combination. The term environment is used in its broad sense and includes not only the physical environment but also the social, economic, cultural, and other environmental states and conditions.

An example of the environmental influence is the low incidence of tuberculosis in range cattle and the high incidence known to threaten dairy herds. During a visit to one of the best laboratories in India, I was quite surprised to discover that calves used in the production of smallpox vaccine were not tested for tuberculosis. I was informed that this disease was not a problem in India among cattle which were allowed to roam freely over the countryside.

In view of the above discussion, the basic factors which govern the appearance, distribution, frequency, and severity of infectious diseases and epidemic outbreaks may be summarized as follows:

1. A host with many characteristics.
2. A parasite with many attributes.
3. Effectual combination of host and parasite with most disease being classified epidemiologically in accordance with its usual avenue of transmission.
4. An environment which exerts an influence on each of the above factors and in turn is subject to major modifications, particularly by man in his efforts to improve living conditions and provide the comforts of life.

Based upon the above concepts, epidemiology of infectious disease may be defined as that field of medical science which is concerned with the relationships of the various factors and conditions which determine the origin, frequencies, and distributions of infectious disease.

Epidemiology draws upon many areas and fields of scientific knowledge for information which will help reveal the natural history of disease processes. Biologic sciences contribute much to the understanding of host-parasite relationships; the same can be said of social sciences which help to define man, his behavior, and culture. Physical sciences clarify the significance of many environmental factors, and the field of mathematics helps in evaluating the significance of observations made by the epidemiologist.

Because epidemiology calls upon resources from such a wide variety of sciences, it is rather obvious that an epidemiologist cannot be defined in terms of scientific background. An epidemiologist is one who devotes his scientific training and technical knowledge to the task of bringing about a better understanding of the natural history of disease for the purpose of disease prevention and control. Thus while the epidemiologist is usually highly qualified in some professional field such as human or veterinary medicine, dentistry, nursing, engineering, sanitation, bacteriology, mathematics, entomology, or parisitology, whether or not a person trained in any of these fields is classified as an epidemiologist will be determined chiefly by how he uses his special training and ability. Often the study of the natural history of a single disease demands the knowledge and understanding of many areas of science, and an epidemiologic team is required for certain kinds of investigations. For example, during World War II, a group of scientists were sent to upper Burma to make an epidemiologic study of scrub typhus fever, which was a hazard to troops in that area. This team included physicians of human and veterinary medicine, bacteriologists, entomologists as well as representatives of a number of disciplines. On the other hand, some of the classical studies in epidemiology have been made by a single investigator. Particularly to be commended is the work of Dr. Pickle, a country physician in England who made great use of the epidemiologic methods in his everyday practice of medicine in a rural area. I am sure that veterinarians, in reading his small volume, will recognize unique opportunities to make use of epidemiologic methods in their daily practice of veterinary medicine. I would like to leave with you the concept that the epidemiologist is one with a curiosity about disease genesis and with a basic interest in disease prevention and control.

When the workshop of the epidemiologist involves the study and prevention of diseases as they occur in the community, he is often referred to as a field epidemiologist. If the epidemiologist uses a laboratory or animal colonies to study the natural history of disease he may be called a laboratory or experimental epidemiologist. Other epidemiologic methods frequently used in studying the natural history of disease are the historical method and the statistical method.

The duties and responsibilities of the field epidemiologist are primarily concerned with disease prevention and control. The preventive role of the epidemiologist is of major importance and should be expressed by a positive action. Instead of waiting for outbreaks to

occur so action can be taken, his energies should be devoted to the prevention of circumstances conducive to disease outbreaks and the initiating of programs which will reduce if not eliminate conditions favoring disease spread.

Your program designed to eradicate tuberculosis through aggressive and yet well designed and scientifically based action exemplifies the ideal practice of epidemiologic principles.

EPIDEMIOLOGY OF BOVINE TUBERCULOSIS FROM A NATIONAL STANDPOINT

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The importance of epidemiology in dealing with tuberculosis has received increased attention in recent years, and it is especially important that additional stress be placed on this facet of the eradication program.

The various factors at each premise where reactors are found must be thoroughly investigated and effort directed toward disclosure and removal of the sources of infection. In studying these factors, consideration must be given other species of animals, as well as man, as a potential source of infection.

Equally important to the eradication program are the tracing to the herd of origin animals that show lesions of tuberculosis on regular kill at the slaughtering plant; the tracing of the origin of animals responding to the tuberculin test; and the followup of those exposed in infected herds.

The effectiveness of these tracing procedures is borne out by the fact that data accumulated over a period of 5 years show that 19 percent of all lesion reactors were found in 7.6 percent of the animals tested. These animals represented 0.38 percent of all cattle tested. These data are illustrated in figure 1.

The finding of such a high percentage of lesion reactors in a relatively small group of cattle may be explained by the fact that many herds have developed heavy infection before lesions of tuberculosis are discovered on the killing floor. Careful testing procedures should continue to detect many infected animals in the early stages of the disease before the herd develops a high degree of advanced infection. It should be emphasized, however, that these tracing procedures must be carried on as an adjunct to other phases of the program rather than as a substitute for any of those procedures.

Figure 2 indicates where the majority of the tuberculosis reactors during the period 1917-57, inclusive, were found. Of the more than 4

million reactors, 69 percent have come from 9 States and 24 percent from 13 other States; this makes a total of 93 percent from 22 States, while only 7 percent of the reactors came from the remaining group of 26 States.

Figure 2 also shows figures on location of reactors for the period 1955-57, inclusive. In the same 9 States, we have found 75 percent of the total reactors in the United States--an additional 11 percent came from the 13 States--making a total of 86 percent in the same 22 States, and only 14 percent from the remaining 26 States.

It is significant to note in figure 3 that the 9 States where the highest percentage of total reactors were found have also yielded 70 percent of the reactors found as a result of tracing animals found with lesions of tuberculosis on regular kill.

Figure 4 further depicts the value of tracing procedures. This figure shows that routine testing, while still very necessary, results in screening a relatively large number of animals to find one reactor, as compared to tests made after tracing diseased or exposed animals. This shows the number of animals tested, for various reasons, to find one reactor.

When we get into retesting quarantined herds and tracing actual cases to their sources, we are working closer to the disease and we get better results in locating infected animals.

Illustrated in figure 5 is the number of cattle tested to locate one reactor as a result of routine testing compared to testing cattle as a result of tracing animals showing lesions of tuberculosis on regular kill to their herds of origin.

Figure 6 shows the complicated path followed from a slaughterhouse in the State of Ohio. An Ohio owner sold his entire herd of 88 Angus cattle in July 1956, anticipating that all would go immediately to slaughter. The animals were taken to an auction sale where 48 went to 12 different slaughtering establishments. Federal inspectors at one plant and municipal inspectors at two plants reported five carcasses as showing lesions of tuberculosis. Forty of the 88 animals were sold to 3 farmers and 3 dealers.

Tests on these premises and 4 additional farms to which dealers had sold some of the cattle revealed 23 reactors on 7 premises. One purchaser who had a reactor sold two animals in violation of the State quarantine on his herd, and was placed under $500 bond. This case illustrates how rapidly animals from infected herds can be scattered. It also illustrates how rapidly tracing can be done--from sales of the herd on July 5, only 22 days elapsed before the last animals from the herd had been located and tested. Had not this prompt and thorough tracing been done, this herd might have been so scattered that an incalculable amount of damage would have resulted. This example also illustrates what may happen when animals are consigned to market for slaughter purposes and the market agencies may find it profitable to divert a portion of the

consignment for dairy, breeding, or feeding purposes. Similar diversions may occur when animals are consigned for slaughter from quarantined herds unless preventive measures are employed.

The relationship of animal types of tuberculosis and the necessity for a broad eradication program are illustrated by cases in which the tracing of swine that have shown lesions has resulted in locating premises with a high incidence of tuberculosis in cattle, swine, and poultry.

As shown in figure 7, 10 swine from a lot of 132 were reported with tuberculous lesions in Albany, N.Y., in April 1955. The tracing of these animals led to the testing of cattle, swine, and chickens on a farm in the Midwest, with the following results: 25 cattle tested--4 reactors; 13 swine tested--13 reactors; 175 chickens tested--106 reactors.

Figure 8 provides information regarding swine showing lesions of tuberculosis on regular kill. In 1924, approximately 54 million swine were slaughtered and of that mumber some 8 million carcasses were retained owing to lesions of tuberculosis. Of the 8 million retentions, 225,000 whole carcasses were condemned.

At that time, with bovine tuberculosis very common and because of the direct association of swine with cattle in many instances, it was reasonable to expect that a high percentage of the swine retentions was due to infection with bovine tuberculosis. It is also logical that a high percentage of the condemnations resulted from the bovine type.

From 1924 until 1954, there was a consistent decline in the number of lesion cases, but from then on further decline has been very gradual. With avian tuberculosis still with us and a contributing factor in swine infection, it would appear that the continued infection rate in swine can be attributed largely to avian infection. These data would also support that one of the continuing problems in connection with the testing program in cattle is exposure of cattle to avian tuberculosis.

In the center of figure 9, an animal is depicted which reacted to the tuberculin test made so that the animal could be considered a proper addition to Farm C. There is no record of previous tests on this animal. As this animal reacted, even though it did not reveal lesions of tuberculosis on slaughter, all of the cattle on Farm C were tested 9 days following the test of the animal that did not have a prior record of test. It is interesting to note that on Farm C one additional reactor was found. The original herd of 25 animals was negative. The two reactors and one other animal on Farm C had originated from Farm A, which had passed a negative test in August 1956. The herd had been reduced in size January 1957, when the three animals were sold to Farm C. In testing the three animals remaining on Farm A, one reactor was revealed. Six animals from Farm A had been sold to Farm D and two of the six animals reacted; the original herd was negative. One animal was sold from Farm A to Farm B, which reacted. The original members of this herd, like those on Farm C and D were negative. As a result of following up on a reactor that did not show lesions of tuberculosis, infection was found as a result of tracing on three additional farms.

Figure 10 shows that the carcass of a thin Guernsey cow was condemned by an inspector employed by the Detroit Board of Health in February 1956. The animal was traced through an auction market to an Indiana farm where 33 reactors were found among the 84 cattle tested. In connection with this investigation, it is interesting to note that a lot of 117 swine was slaughtered in a Federal establishment in Detroit where 8 showed lesions of tuberculosis. Three of the eight carcasses were sterilized and one condemned. Eighteen carcasses showed lesions of tuberculosis at Buffalo, N.Y. These swine also had originated from the same Indiana farm. Five of these carcasses were condemned and two sterilized. It will be noted that 12 head of the breeding swine reacted and 10 chickens reacted to the tuberculin test on this farm.

Figure 11 shows a report, dated February 1957 from a California meat inspector, indicating that lesions of tuberculosis had been found in two cows. Tracing led to a ranch in Idaho where a range herd and a purebred herd were maintained. Two tests in 1957 and two tests in 1958 revealed 124 reactors. A high percentage of the reactors were taken from the purebred herd. Forty-three reactors were finally removed from 8 associated herds. In January 1958, a Federal veterinary meat inspector in Utah reported lesions in two Hereford heifers. It was determined that the affected heifers had passed through a feedlot in Idaho and originated from a herd in the same State. A test of that herd revealed 10 reactors. Thus, 177 reactors were removed from 10 herds in a State that had reported only 6 reactors in the previous 5-year period.

Figure 12 indicates that on February 12, 1958, well-marked lesions of tuberculosis were revealed in a Minnesota packing house on necropsy of a Holstein cow bearing an ear-tag number MLSSB T360103. The veterinary meat inspector reported that the animal had been purchased at the local stockyards. It had been consigned from a nearby herd where a subsequent test revealed another infected animal. This herd had contained animals purchased in June 1957 from a herd sold at auction. Animals from the sale were traced to 11 buyers in Minnesota; 8 herds were tested. Twenty-six reactors were revealed in 7 herds. Animals from the same sale had been consigned to two herds in Wisconsin. Five reactors were found in one of the Wisconsin herds. The total results were 31 reactors removed from 8 herds in 2 States.

In closing, it is important to reiterate that the movements of cattle associated with every infected herd must be successfully traced so that every possible focus of infection will be revealed. Considerable progress has been made both in determining the history of reactors and following up on exposed animals as disease control officials have become more familiar with the system of reporting the history and movement of animals to and from infected herds. However, steps must be taken to further improve this system through intelligent and diligent attention to each of the reports received.

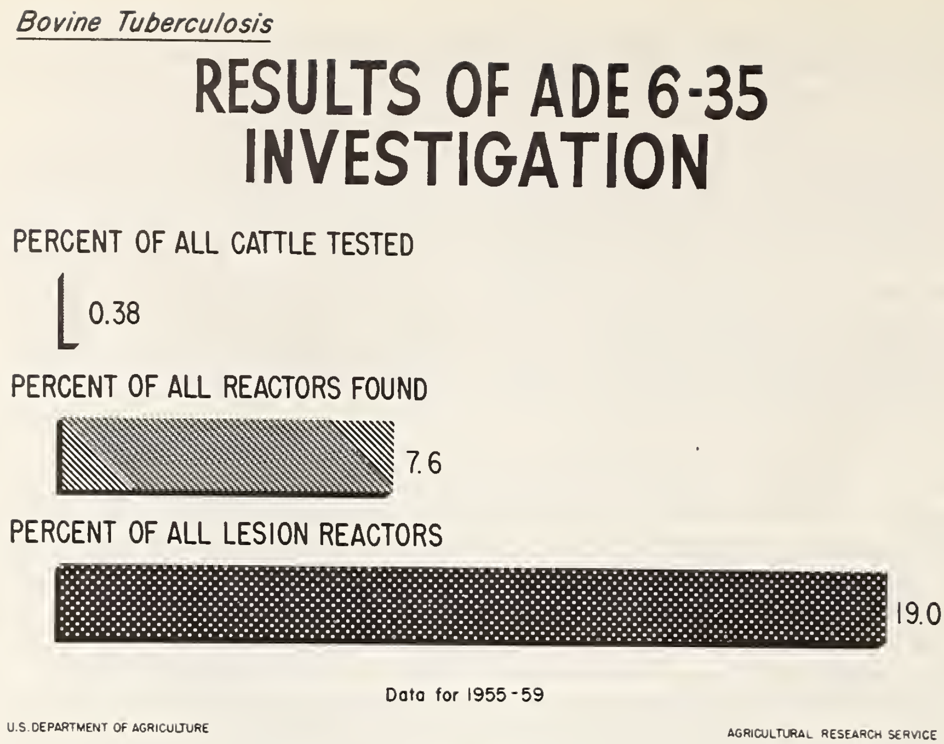

FIGURE 1

BN-11658-x

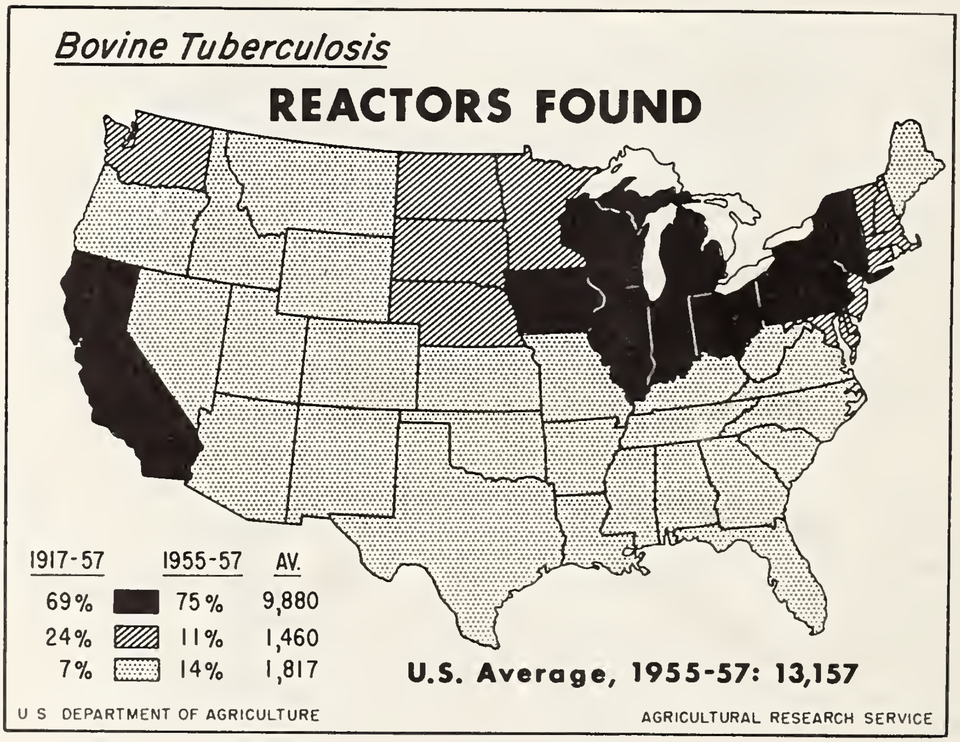

FIGURE 2

BN-11659-x

TB REACTORS FOUND

By Tracing Lesion Cases, Regular Kill

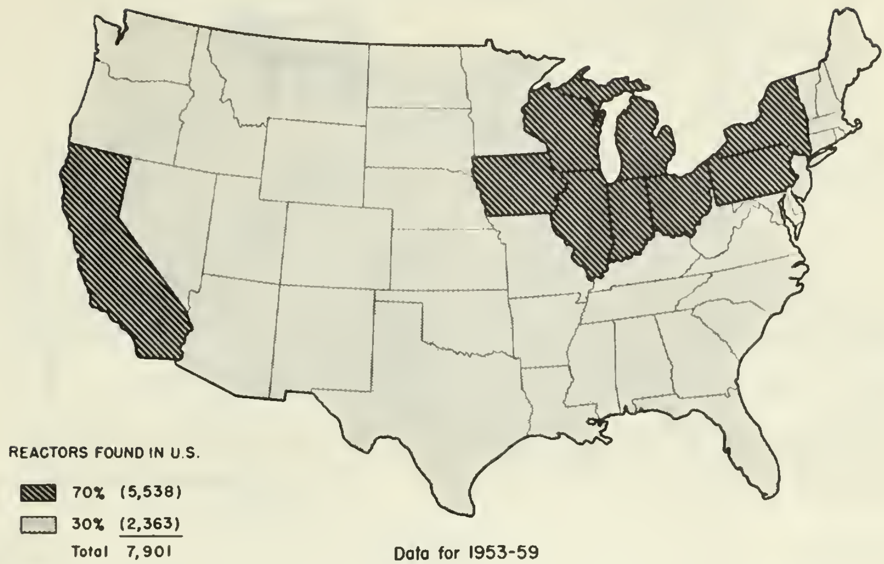

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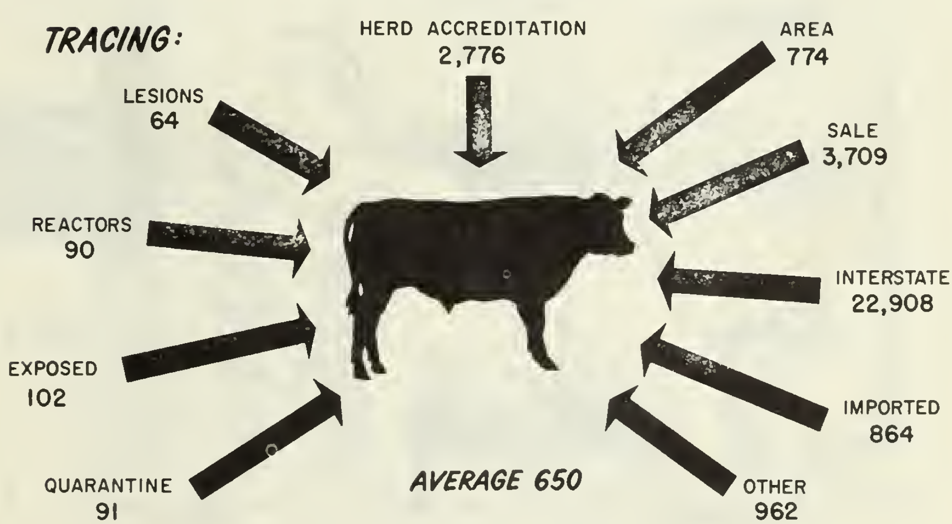
FIGURE 3 BN-11660-x

CATTLE T.B. TESTED

To Locate One Reactor



TOTAL: Cattle tested, 5,302,658 Reactors found, 8,158

Jan. 1 - June 30, 1956

FIGURE 4 BN-11661-x

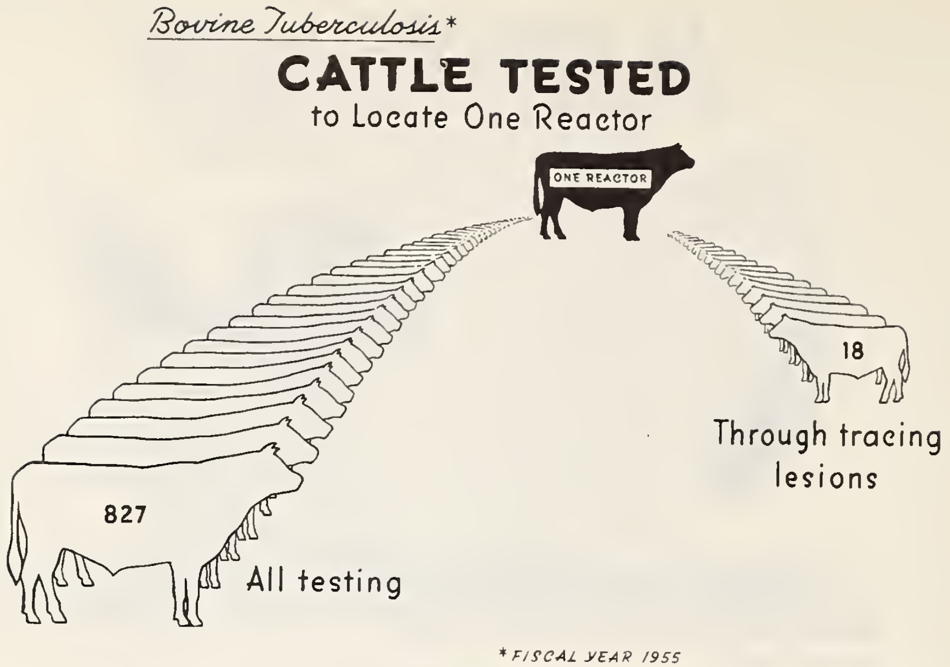


FIGURE 5

BN-11662-x

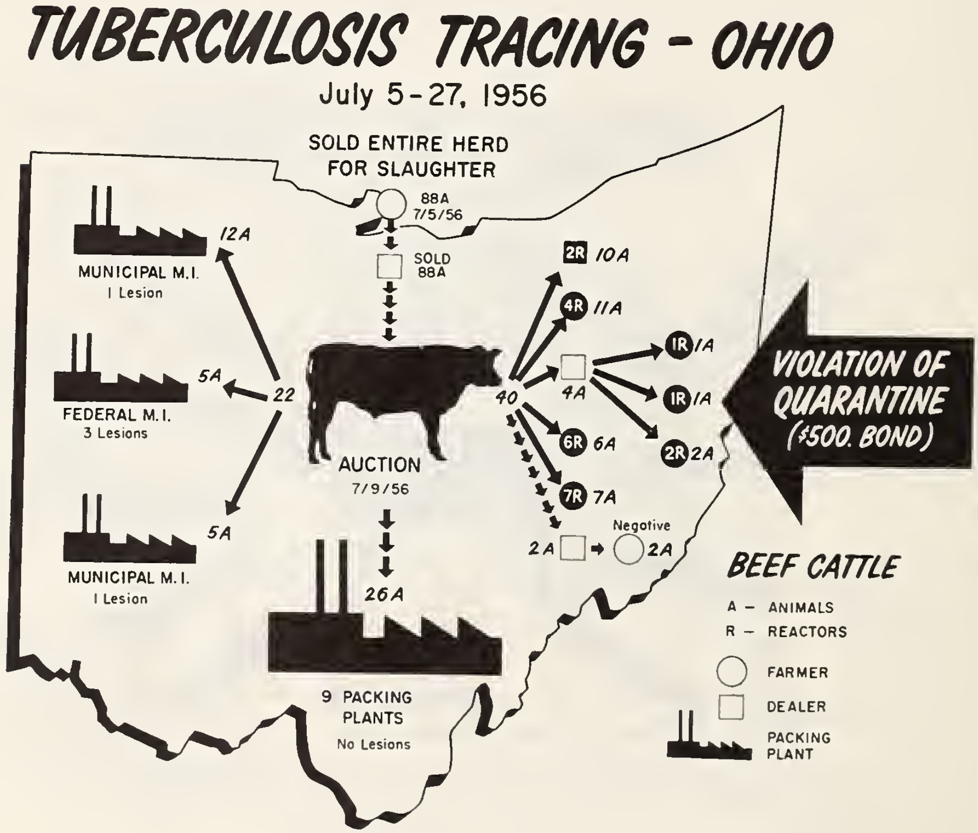


FIGURE 6

BN-11663-x

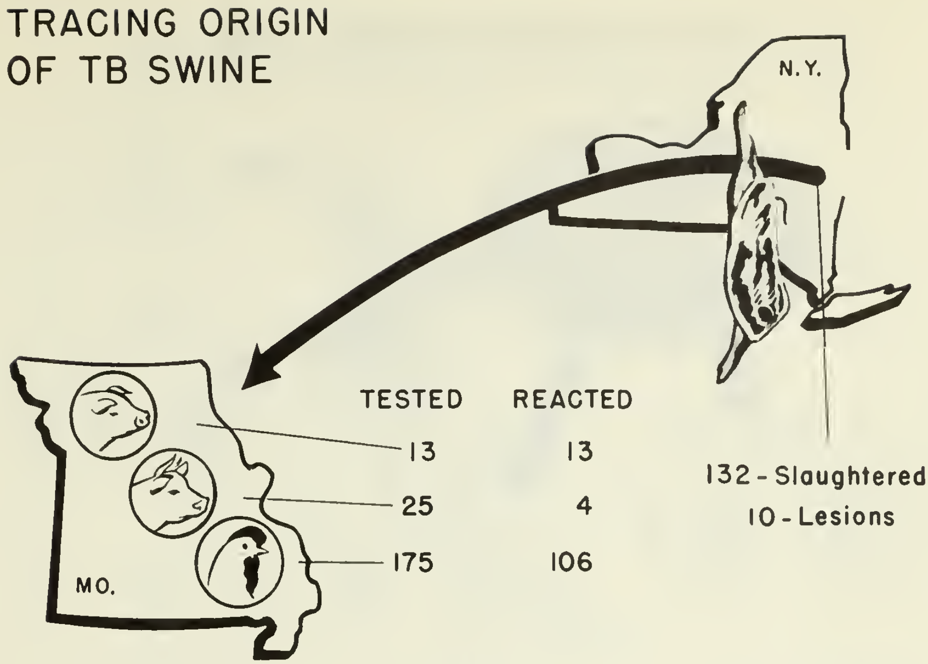


FIGURE 7 BN-11664-x

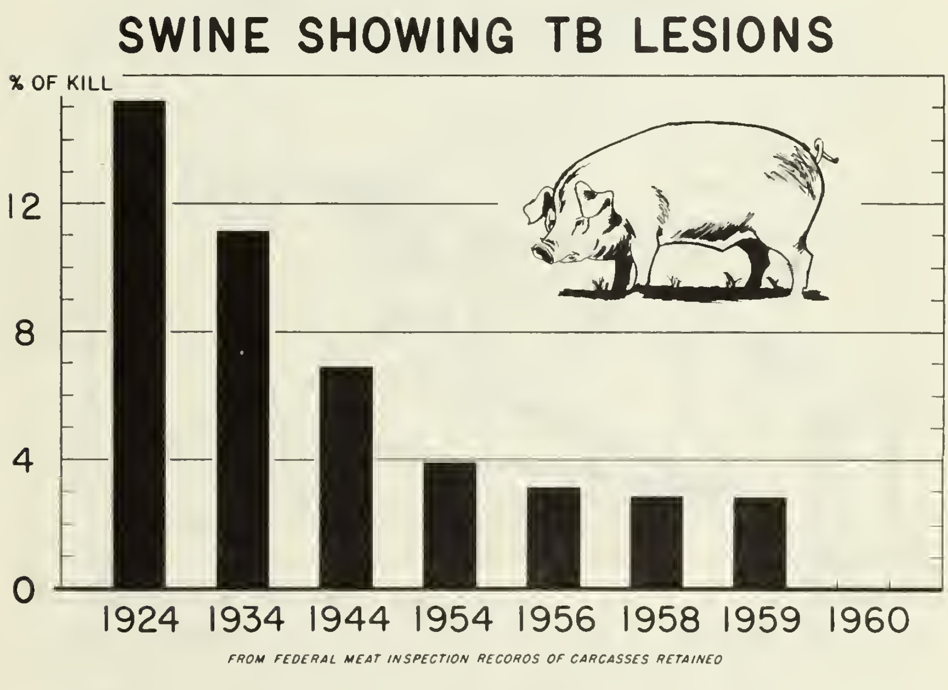


FIGURE 8 BN-11665-x

TB REACTORS DISCLOSED

By Tracing Origin of NVL Case

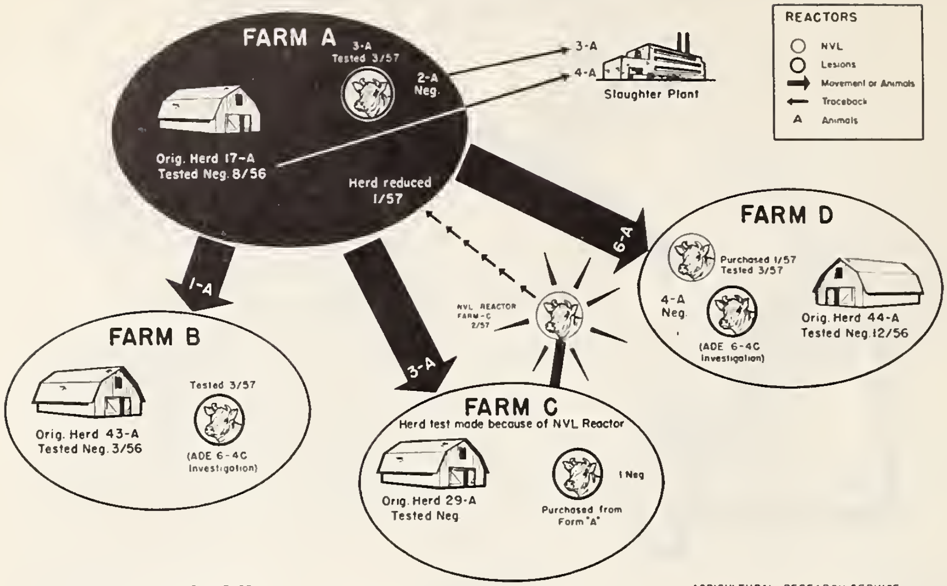


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FIGURE 9 BN-11666-x

TUBERCULOSIS TRACEBACKS

Lesion Cases Regular Kill

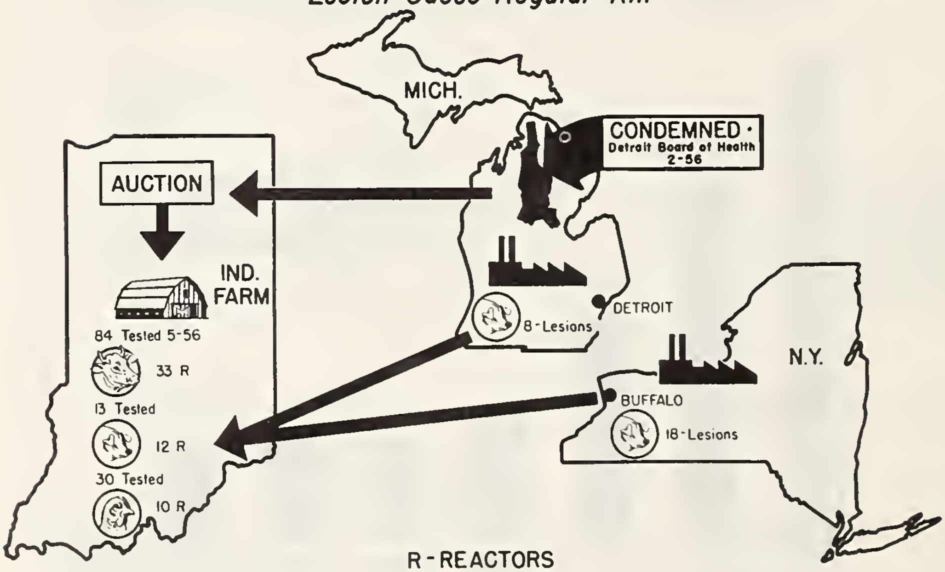

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FIGURE 10 BN-11667-x

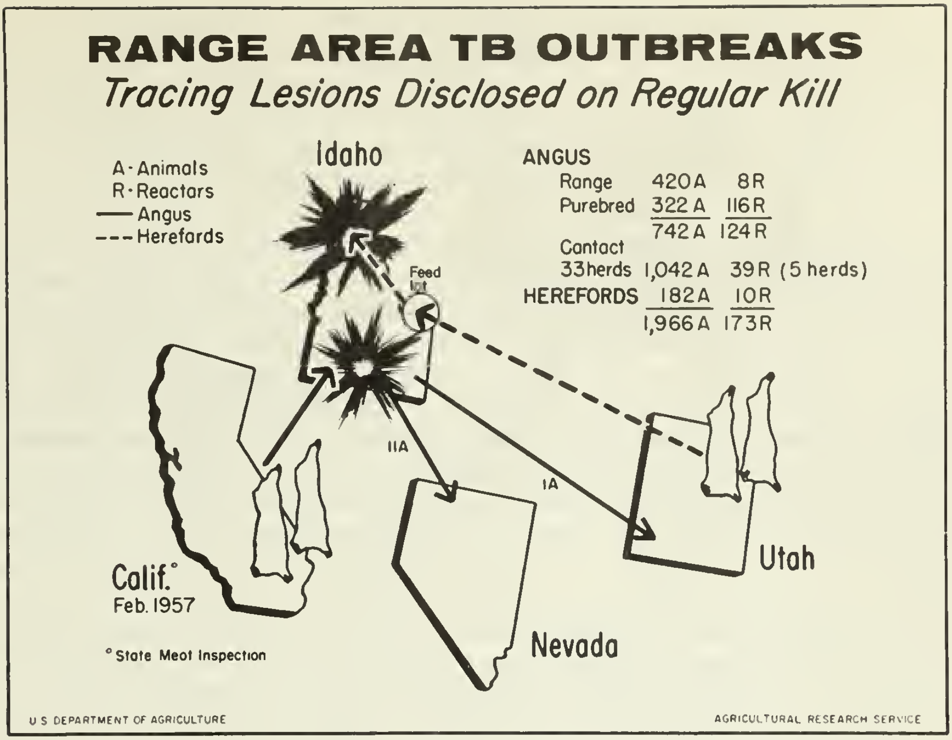

FIGURE 11 BN-11668-x

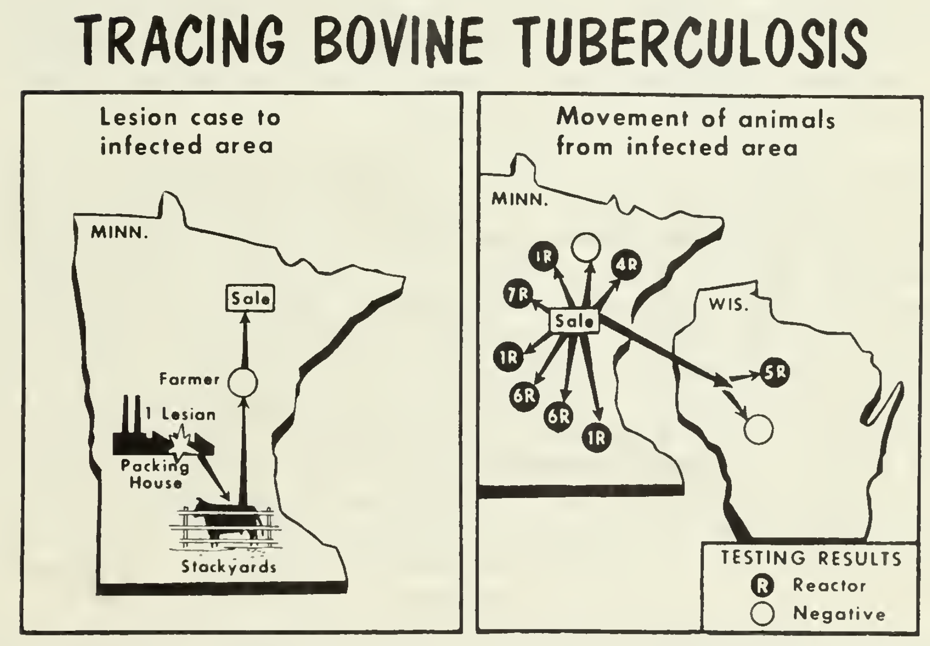

FIGURE 12 BN-11669-x

EPIDEMIOLOGICAL CONSIDERATIONS OF TUBERCULOSIS IN ANIMALS AND MAN

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A relationship between tuberculosis in animals and man has been recognized since antiquity, and numerous measures have been employed to prevent transmission of this disease. Religious restrictions against eating the flesh of swine perhaps were in part founded upon the fact that swine are susceptible to all three types of *Mycobacterium tuberculosis*. Throughout history many other efforts to prevent human infection with the tuberculosis organism from animal sources have been attempted. Control measures have been changed from time to time as knowledge of the epidemiology of tuberculosis has been accumulated. The early prohibitions against eating the flesh of diseased animals were based upon observations of the similarity of the symptoms of tuberculosis, and upon gross pathological changes, in animals and man. We can be rather certain that the observations of involvement of the cervical lymph glands and lesions in other areas, closely associated with the alimentary tract, were convincing evidence that tuberculosis was contracted from animals through ingestion. Isolation and identification of the tubercle bacillus by Koch added to this evidence. The way was thus opened for showing in later years that the causative organism was transmitted to humans through contaminated milk, and led to requirement that milk be pasteurized. Health agencies seeking further safeguards for human health struck at a known source of infection by requiring that market milk originate only from cattle which were free from tuberculosis. As a companion safeguard, meat inspection services removed from commercial channels all meats recognized as being unsafe for human consumption. However, neither pasteurization nor meat inspection had any effect upon human pulmonary infection of bovine origin.

Sigurdson (3) 1/ in Denmark has shown that the amount of human pulmonary tuberculosis of bovine origin is directly related to the amount of contact between humans and infected cattle. He has also shown that bovine infections in man are in direct relation to the amount of tuberculosis in the cattle with which persons have been in contact. He has assumed that the open bovine pulmonary tuberculosis in man arises from inhalation infection during work of the patients in cattle barns, an observation supported by the high incidence of bovine-type infection, as compared with human-type infection, in rural patients between the ages of 15 and 30 years. This age group comprises the "age of hired help" in cattle barns for many of the rural population in Denmark. The relatively mild bovine infections in humans appear to have developed both as alimentary and as aerogenous infections.

1/ Numbers in parentheses refer to Literature Cited, at end of this paper.

Sigurdson's studies (3) showed that the course of disease resulting from either the bovine-type or human-type organism is essentially the same. The subjective symptoms of pulmonary disease are alike in both types of infection. No essential differences were observed in the temperature, sedimentation rate, tuberculous complications, or roentgenographic changes in the lungs. The case fatality rate for the two groups of infection was fairly alike and the prospects for recovery were practically the same. It was agreed among Sigurdson's collaborators that the bovine and human tubercle bacilli are equally virulent for man. Sigurdson concluded that without complete eradication of tuberculosis from cattle this disease will never be eradicated in man.

Economic considerations as well as concern for human health have brought about measures for the eradication of bovine tuberculosis in this country during the past four decades. The organized program for bovine tuberculosis eradication in this country has been an outstanding example of successful disease control, and this success is recognized throughout the world.

Reduction of tuberculosis in our cattle has been an important factor in reducing the incidence of bovine-type tuberculosis in man to an all-time low in this country. The last reported confirmed case occurred in 1957 in a rendering plant-slaughterhouse worker in Massachusetts (4). The most recent reported proven case of the disease in children was in 1954 in a 6-year-old Ohio farm boy, who had a cervical adenitis identified as tuberculous on histopathological examination (4).

Since 1947 there have been 12 episodes involving humans with identified bovine tuberculosis. One involved children who showed a sharp increase in positive tuberculin reactions while using raw milk from a diseased herd. The other 11 episodes were clinical cases. One of these was a tubercular mother with a 2-year-old child diagnosed as having tubercular meningitis. Cultures were not made from this child, but pulmonary-bovine type tuberculosis was confirmed in the mother. In addition to this woman, six cases were confirmed by identification as *Mycobacterium tuberculosis* var. *bovis*. The remaining three cases were associated with diseased cattle; diagnosis was confirmed in two cases by histopathological examination and by isolation from the other of *M. tuberculosis* which was not typed. Seven of these 11 clinical cases occurred in adults, five of which had lesions in the chest cavity, one in the spine, and the other in the cervical lymph nodes. In contrast, there were no pulmonary lesions among the four children; three had cervical node involvement, and the other had meningitis. All episodes involved persons residing in the eastern half of the United States. All were native born except two, one of which had been confined for 3 years in a German prison camp; the other had been raised on a Canadian farm. The child exposed to his tubercular mother indicates human-to-human transmission of the bovine-type organism, although this was not proved by isolation and identification of the organism in the child. One episode was in an abattoir worker who could have been exposed to the disease in either cattle or hogs. Six cases were exposed to cattle

either directly or through milk. The exposure of the remaining cases is unknown.

The paucity in this country of human tuberculosis due to the bovine-type organism reflects the diminishing contact with infected cattle. But when considering the epidemiology of a disease, all factors possibly influencing the incidence must be taken into account. Many factors have influenced the reduction of tuberculosis in humans, and it is most difficult to determine which has been most important. Price (2) points out that the reduction in human mortality from extrapulmonary forms of tuberculosis (the form usually associated with the bovine type in this country) parallels the eradication of tuberculosis in cattle. Anderson (1) considers such a parallel is not convincing when one notes that the reduction in mortality from extrapulmonary forms also parallels the reduction in mortality from pulmonary forms. Exact data are not available, so the question of whether we have less scrofula and less tuberculosis of the spine, bones, and joints in the United States because of less tuberculosis in cattle or because of factors which have brought about lowered pulmonary tuberculosis death rates remains a matter of speculation. A higher level of socioeconomic conditions, and control measures such as aggressive case finding, isolation of cases in sanatoria, and improved, more specific treatment, would certainly be expected to influence the downward trend of incidence in all forms of tuberculosis.

Whatever the influence other factors have had upon bovine tuberculosis in humans, no one can minimize the excellent success of the bovine tuberculosis eradication program in case finding and removing these sources of human exposure. In fact, this program has been so effective, human bovine type infection in this country attracts very little specific attention from public health agencies and tuberculosis control officials. What interest there is usually is more of an academic than epidemiological nature.

Case finding techniques used for both humans and cattle have been most productive but now have reached a stage of diminishing returns and the value of these techniques needs a reevaluation and the techniques themselves perhaps need alteration. Repeated mass chest X-rays were used successfully for many years to locate cases or suspect cases of human tuberculosis, but last year the Department of Health, Education, and Welfare recommended that this practice be discontinued and that the tuberculin test be used instead in groups where experience has shown a low incidence of cases. It was also recommended that when tuberculin reactors were found other case finding techniques be used for investigation of the case and circumstances surrounding it. In making the recommendations, consideration was given to the number of cases found by X-ray, radiation hazards associated with regular examination, and the stage

of disease when lesions would show on the film. Many false positives were being reported because of the difficulties encountered in interpreting shadows on films and in obtaining uniform results from a large number of people reading the films.

Generally similar difficulties are being encountered in the best case finding technique used on cattle. All of you are familiar with the problems of the tuberculin test and know it is not infallible in cattle. The failures and inaccuracies of any test for a disease are relatively unimportant when the disease incidence is high. However, as the point of eradication is approached, all of the factors influencing the accuracy of case finding and identification of infection become proportionately greater and present problems requiring a solution. The tuberculin test technique has been highly effective in reducing the spread of tuberculosis among cattle but the persistance of infection and the increasing proportion of reacting cattle which show no sign of disease raise the question of the need for supplementing the tuberculin test at this stage of eradication with other investigative procedures and techniques.

It would appear that there are many facets in the epidemiology of tuberculosis in this country which need elucidation and require the intensified cooperation of all agencies having an association with the disease. Case finding by testing with tuberculin may not be adequate to locate foci of infection soon enough to prevent propagation of the causative organism. All reasonable methods of finding cases should be used and intensive efforts made to eliminate these. Sources of cattle infection in other species may require investigation; certainly the nontuberculous reactor to tuberculin presents a problem which we can expect to become more vexing as the incidence of tuberculosis is reduced.

Assistance in finding and investigating foci of infection or reactors should be available from health departments familiar with the health status of humans in contact with livestock and be available from meat inspection services. Prompt reporting of tuberculosis in rural groups, in stockyard workers, or in slaughtered animals will give an opportunity for early investigation of conditions surrounding these cases.

An attempt was made recently by Dr. Ranney and myself to determine the cooperation between State health departments and State veterinary services in investigating circumstances surrounding cases of tuberculosis or cattle reacting to the tuberculin test. Information was obtained from 31 States; 29 of these reported some type of cooperation between the two agencies. However, the extent of cooperation varied greatly and was difficult to evaluate. In nine of the States reports of human and animal cases were exchanged routinely. In five, regular reports were sent only by the veterinary services to the State health departments. In two States, reports were submitted by the veterinary services to the local health departments where cases were found in cattle.

Occasional special reports were exchanged in some States, and in two States personnel from the public health and veterinary services participated in annual work of planning conferences.

It was not possible to determine with any degree of accuracy the extent of investigations which followed reports of cases. Apparently this depends upon the availability of personnel and their interest. There have been so few human cases of tuberculosis of known bovine origin that there seems to be no widespread enthusiasm for extensive investigation of individual cases or of persons associated with reactor cattle.

Although many investigations in cooperation with health departments would yield negative results, it would seem they are necessary as a definite source of epidemiological data needed for complete control of tuberculosis and needed for an understanding of the increasing proportion of tuberculin-reacting cattle which show no lesions. It would seem reasonable to expect that closer cooperation and active participation by human and animal health agencies in the investigation of tuberculosis cases would be beneficial and rewarding to both agencies.

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LABORATORY ASSISTANCE TO EPIDEMIOLOGY

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Behind every successful epidemiological endeavor are the essential support services provided by the laboratory. This is especially true in investigations of tuberculosis in domestic animals and birds. The laboratory serves as a focal point for developing and providing supplementary information on which is based a sound conclusion of field findings. The tuberculosis unit of the ADE Diagnostic Laboratory at Ames,

Iowa, is presently engaged in the detailed examination of tissues from animals classified as no-gross-lesion (NGL) reactors in the field. Several types of acid-fast cultures have been isolated from skin lesions submitted to the laboratory by epidemiologists working in the field. These cultures may grow as highly pigmented growth on the surface of slants that are used to culture Mycobacterium tuberculosis. Some are yellowish in color, and in many respects resemble true tuberculosis cultures. However, as a rule, they grow more rapidly than M. tuberculosis. While they are acid-fast, they may reveal wide variation in morphology when reviewed under the microscope.

Several types of atypical or saprophytic mycobacteria have been isolated from human subjects, and some of these have been found to cause reactions to the tuberculin skin test. It might be of interest to review some of these types. It is one of the goals of the work at the ADE Diagnostic Laboratory at Ames to determine if similar organisms may be a factor in NGL reactions to the tuberculin test in animals.

Mycobacterium balnei is one type of atypical mycobacterium that was first isolated in the Scandinavian countries about 1952. It was recovered from a tuft of hair caught in the outlet of a local swimming pool. It was characteristic of 11 other strains of the same organism recovered from humans during an epidemic. The conditions under which this organism was isolated closely parallel those on the average dairy farm. NGL reactors may have skin lesions, and acid-fast organisms have been observed on microscopic examination of tissues from some. As animals received bruises or abrasions, such organisms may have an opportunity to enter the skin. Close observations in this regard should be made by the epidemiologist in the field. These should be included in the complete report on individual cases as supplied to the laboratory.

Mycobacterium ulcerans is another atypical mycobacterium that has been recovered from humans. Consider how its history closely parallels the situation in our animal population: "Isolated in 1948 from an ulcer near the middle of the outer surface of the left forearm of a male dairy farm worker, age 26."

Another isolation of this same culture read: "Isolated in 1948 from an ulcer on the lower third of the left shin of a farm woman, age 51." Both of these isolations were made in Australia. This organism grows best on a special medium called Herrolds Egg Yolk medium. This culture medium is not routinely used by most laboratories, but is now incorporated in the laboratory procedures at Ames, along with several others.

There are other atypical mycobacteria that have been isolated from humans, including the so-called photochromogens that produce pigment in the light only,the scotochromogens that produce pigment in light or darkness, and the non-photo-chromogens that do not produce pigment. There are also the rapid growers and various soil acid-fast bacilli.

As the laboratory gains additional experience in the isolation of these organisms and their classification and identification, it may provide a real breakthrough of information and data to aid epidemiological activities. This is an area of very fruitful study at the present time. Laboratory techniques can obviously play an important role in demonstrating the etiological agent. Inadequate laboratory techniques could render negative results when the animal is actually infected by atypical acid-fast bacilli or typical tubercle bacilli.

There are, as everyone appreciates, limits to studies that can be undertaken in any laboratory. The laboratory working in support of field programs for the eradication of tuberculosis must go beyond the isolation of an atypical acid-fast culture from a lesion that may be submitted. We need to know much more. Thus, the laboratory staff must often extend their routine findings to further investigations. In this case it is of interest to prepare allergens from nontuberculosis cultures that may be isolated from lesions. These, in turn, must be used under laboratory and field conditions to determine the response of animals of known infection status. Studies should also be conducted to determine if the cultures will sensitize animals to react positively to the tuberculin test.

There is presently much interest in serological laboratory tests for tuberculosis in cattle. The hemagglutination, gel precipitin, and complement-fixation tests are under study both in the human and, to a more limited extent, in the veterinary field. These tests used as an adjunct to the tuberculin skin test could have wide application, particularly in areas where NGL reactors are a problem. Here also the laboratory is in an important position to aid epidemiological studies. It would be of advantage to have available large serum banks made up of samples collected from animals from which tissue specimens are submitted. These would provide a large number of serum samples from animals on which complete culture results are available. The testing of such serum samples with various serological procedures will provide valuable information on the possible application of serological tests for tuberculosis.

The laboratory also has an important responsibility in the actual training of epidemiologists engaged in field investigations. The ADE Division has recognized this need, and is affording laboratory training to selected personnel as time and funds permit. Plans are to expand this training service at the new National Animal Disease Laboratory now under construction. Training services presently utilized include those of the Communicable Disease Center of the U.S. Public Health Service, the Trudeau Foundation, and State diagnostic laboratories with a special interest in tuberculosis.

It is essential in epidemiological work to insure that properly trained individuals are assigned where specimens are to be collected. The laboratory has the responsibility to train these individuals in the proper collection, handling, packing, and delivery of specimens to the laboratory. They should be instructed in the importance of proper technique in making the collections and in selecting material that is most likely to yield information. If epidemiologists are

familiar with the actual procedures used in the laboratory in examination of specimens, they will do a better job in the field in making collections. Thus, the epidemiologist can very profitably spend a short period of time actually working with the laboratory staff.

Records are very important in field epidemiological studies. Data on each animal should be available and each specimen properly labeled so that identification can be readily made. Because of the nature of slaughtering plant operations it is not always feasible to use absolutely sterile techniques in collecting each specimen. Specimens should be collected carefully, kept cool, not contaminated after collection, and identified accurately. If suspicious lesions are noted in any organ or gland, that organ or gland should be collected for laboratory study. Specimens should not be incised any more than necessary.

When specimens are received in the laboratory from field epidemiologists, they are subjected to careful study before a report is sent to them. Culture tests and animal inoculations of necessity take time and often tend to tax the patience of the field epidemiologist. Tuberculosis cultures grow slowly and may require 8 to 10 weeks of incubation before they can be accurately identified. Animal inoculation, which is the best method for demonstrating tuberculosis organisms in specimens, may require 6 weeks or longer. Typing of isolated cultures involves the inoculation of chickens, guinea pigs, and rabbits, and requires about 6 to 8 weeks for completion.

To offer an immediate preliminary report to the epidemiologist, the laboratory prepares smears of submitted tissues and also histological sections. These tissues are stained and examined microscopically for acid-fast organisms and pathological changes. The reports of these examinations are usually the first received by the epidemiologist, and are of most immediate assistance to him. Thus, cultures and animal inoculations, while very valuable in long-term studies of animal tuberculosis, offer the epidemiologist little immediate information. The laboratory staff must be well trained in the pathology of tuberculosis and be able to differentiate it from similar conditions such as the granulomas.

In long-term studies of field problems in tuberculosis eradication the laboratory must work as a team with the epidemiologist. Field studies of various tuberculins, tuberculin fractions, johnin, and PPD, while actually administered in the field by the epidemiologist, require that he be in close consultation with the laboratory staff. The laboratory staff and the epidemiologists must plan such studies together through careful discussions. The laboratory staff must prepare, standardize, and supply the materials to be investigated. Data collected from such studies must be analyzed and interpreted as a cooperative effort between the epidemiologist and the laboratory staff.

In offering new aids to the epidemiologist in this changing field of science the laboratory must apply, as well as develop, newer and better methods of diagnosis. One such aid is the use of fluorescent antibody techniques to identify TB organisms in diseased tissues. Also

newer media, which promote the more rapid growth of TB organisms, should be studied and applied. Newer methods of typing mycobacteria through the use of chemical procedures also warrant additional study and application.

In field studies of the factors influencing the persistence and spread of tuberculosis, the epidemiologists may make collections of soil, feed, and various other materials that might serve as a carrier in the spread of tuberculosis. When such materials are received by the laboratory, they may be cultured, examined microscopically, or inoculated into animals for the determination of their bacterial flora. The accumulation of information on the various extraneous organisms in different soil types can materially aid our understanding of those types of soil and soil organisms, that may contribute to problems in the field.

The role of the laboratory in supporting the efforts of the epidemiologist is not a small one. Where there is an epidemiologist, if he is to be successful he must be supported by a qualified team of men in the laboratory. Toward this end the Animal Disease Eradication Division is maintaining a trained staff of workers at its Ames laboratory, is expanding its epidemiological teams, and is encouraging more State laboratories to undertake additional work in tuberculosis where their staff and budget permit.

VI. REMOVE CAUSES OF ASSOCIATED SENSITIVITY

PARATUBERCULOSIS

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Johne's disease has been reported from many countries throughout the world. It has been diagnosed in more than 500 herds in the United States, and evidence at hand indicates that it is continually spreading and probably exists in every State. However, since Johne's disease is not a disease of human beings, it has received only a fraction of the attention paid to tuberculosis.

Additional infected herds probably would be found if more testing were done and the disease reported each time it was diagnosed. Estimates of losses from Johne's disease in infected herds may involve uncertainty since they so often depend on a herdsman's memory as to actual cause of death; losses, however, may be greater than the owner thinks. Actual loss encountered in a herd of purebred Guernseys consisting of 100 adult animals and 67 young stock was studied by USDA workers for 12 months. No attempts at control were made. Of 18 animals culled during the year, 7 were eliminated because of Johne's disease. That's as many head as were removed for reproductive disorders and more than the total taken out for all other reasons. Salvage for these 7 animals was estimated to be $1,300 less than their value as dairy animals.

Johne's disease probably shortened each of their lactation periods as much as 6 months. Milk was bringing about $25 a head monthly over feed costs for each animal, so the total milk production loss amounted to a thousand dollars. In addition to these losses, the owner was advised against selling surplus heifers for dairy purposes because of the diseased condition of the herd. This resulted in an additional loss. Such losses continue year after year unless the disease is brought under control. The impact of Johne's disease on the agricultural economy of a number of European countries is so severe that the European Productivity Agency recently sponsored a seminar on the control of the disease which was attended by personnel from 10 countries belonging to the Organization for European Economic Cooperation. The report published by this group is the first positive step toward group effort against Johne's disease.

Johne's disease is described as a chronic infectious disease of cattle, sheep, and goats, characterized by inflammation of the intestines and a recurrent diarrhea that may persist for months. Ruminants are the only animals that have been found to be susceptible to infection.

Johne's disease is caused by a small rod-shaped acid-fast bacillus about 0.5μ in width and $1\frac{1}{2}\mu$ in length, named Mycobacterium paratuberculosis. This organism has staining characteristics identical with those of the tubercle bacillus, the Ziehl-Neelsin staining procedure being used routinely. The bacteria attack the intestinal wall, where they are usually found in clumps. They are most often found in the posterior part of the small intestine, ileocecal valve, the large intestine, and the rectum, although they may also be found in the adjacent mesenteric lymph glands.

Mycobacterium paratuberculosis is very difficult to culture on artificial media. A detailed discussion of culturing techniques is not within the scope of this paper. Briefly, the infected tissue is minced and mixed with antiformin or an acid to destroy all except acid-fast bacteria. The treated tissue is placed on media containing dead acid-fast bacilli or extracts of them. Other ingredients of the media are variable depending on the bacteriologist who is attempting the isolation. An entirely satisfactory medium for cultivation has not as yet been developed.

Chemical analysis shows that M. paratuberculosis, like other mycobacteria, contains, a relatively large amount of fat and wax. About 50 percent of the weight of fat-extracted bacilli is protein. In addition, they contain carbohydrate, phosphorus, magnesium, sulfur, potassium, sodium, calcium, and iron in measurable amounts.

The bacillus survived 270 days when stored in tap water, pond water, or distilled water, and 246 days in bovine feces, and only 7 days in bovine urine. It has been found to survive 47 months in a desiccated state and at least a year in a cold storage freezer at $-14^{\circ}C$. Bacilli placed on filter paper and exposed to sunlight through cellophane were viable after 65 hours of exposure but not after 100 hours of exposure.

The effect of various disinfectants has also been studied. Cresylic disinfectants are as effective against M. paratuberculosis as they are against M. tuberculosis. In dilutions of 1:32 they destroyed M. paratuberculosis in 15 minutes. Sodium orthophenylphenate destroyed the bacillus in 15 minutes in a dilution of 1:200. This product has no objectionable odors and is not expensive to use. Products that did not destroy the bacillus were: Six percent sodium hydroxide solution and quaternary ammonium solutions containing 1:250 available quaternary. It required 14,000 ppm. available chlorine to destroy the bacillus. This is about 150 times the usual concentrations used for disinfecting premises.

The bacillus is disseminated in the droppings of infected animals, and susceptible animals become infected by ingesting the contaminated dropping in the feed and water. Evidence is also accumulating that indicates infection can pass from a dam to unborn offspring.

The disease is not readily transmitted from herd to herd except through traffic in diseased animals, as was shown by a survey conducted on 170 diseased herds. In a number of herds in which the survey was made, the method of introduction was unknown; but where the information was available, in all instances except two, the disease had been introduced by the purchase of infected animals. The two exceptions were instances in which drainage was suspected from infected premises in the vicinity.

Experimental transmission of the disease can be accomplished by feeding susceptible animals infected mucous membranes or injecting virulent bacilli intravenously. Results of infection experiments show that young animals are much more susceptible to the disease than mature animals. It has also been shown that the incubation period is a year or more unless an unusually large inoculum is given.

Clinical symptoms are usually observed in mature animals between 2 and 5 years of age. Symptoms are first observed in females within a few weeks after calving. Males may break at any time during the year, usually after a heavy breeding season. The usual symptoms are a persistent diarrhea without straining, which respnds only temporarily or not at all to the usual treatments for that condition. A rapid loss of flesh and tight skin accompanies the diarrhea; however, the temperature remains normal and the affected animal continues to have a good appetite until the terminal stage of the disease. Occasionally, the diarrhea stops, weight is regained, and the animal shows improvement; but such improvement is usually of a temporary nature and the animal begins scouring again and eventually dies. An alert herdsman may notice a gradual loss of flesh and diminished milk flow a month or more before the actual diarrhea begins.

Johne's disease can be confused with parasitism, malnutrition, acetonemia, and hardware disease, and may be diagnosed as one of these conditions if a hasty diagnosis is made.

Apart from general lesions of emaciation, specific pathological changes are usually confined to the intestine and associated mesenteric lymph nodes. The intestinal wall is thickened and the mucus membrane is found to be abnormally corrugated or wrinkled. Evidence of ulceration or erosion is not found, but mucosa may show areas of congestion. The ileocecal valve may be swollen and congested. Macroscopic post-mortem findings are undependable for diagnosis, because intestines from diseased animals sometimes appear normal.

Histological examination shows the invasion of the infected tissues by large numbers of cells of an epithelioid nature. These lesions may extend into the submucosa. The bacilli may be extracellular or intracellular and may also be found in adjacent lymph nodes.

A tentative diagnoses of Johne's disease can be made by observing typical clinical symptoms of the disease; however, this must be confirmed by obtaining a positive johnin test and by finding the typical small acid-fast bacilli in stained smears prepared from rectal mucosa or feces or histological sections removed from the intestinal wall and adjacent lymph glands. Rectal mucosa can be obtained from a living animal by inserting the arm deep in the rectum and scraping the bowel wall with a fingernail.

Failure to find organisms in smears prepared from rectal scrapings or feces does not justify a negative diagnosis and the animal should be under suspicion and isolated until the cause of the illness has been determined. If the animal is suffering from Johne's disease, it will usually die and the bacilli can be found in smears or histiological sections prepared from the intestine and lymph glands after post-mortem examination.

If the individual attempting to make the diagnosis is not equipped to make a microscopic examination, he should send specimens to a diagnostic laboratory. The specimen should consist of 24 inches of large intestine containing the ileocecal valve and about 12 inches of attached small intestine along with adjacent mesenteric lymph nodes.

Diagnostic agents play an important part in diagnosing the disease. Both johnin and avian tuberculin have been used subcutaneously, intravenously, and intradermally as diagnostic aids. The intradermic test with johnin is less time consuming, as accurate as other methods, and is the test commonly employed by the Animal Disease Eradication Division and State Livestock officials. The test is conducted by injecting 0.2 cc. of johnin into the skin of the caudal fold or the cervical region. The cervical region is more sensitive to johnin than any other skin area of the animal's body, including the caudal fold. A positive reaction consists of a slight swelling similar to a tuberculin reaction 48 hours after injection. The swelling tends to be softer and more diffuse than swelling due to injection of tuberculin.

There is still a recognized need for a more sensitive and specific test for use in herds where Johne's disease is particularly difficult

to eradicate and difficult to differentiate from sensitizations caused by other acid-fast bacilli. Investigations of possible diagnostic procedures have involved allergic and serologic studies. In the development of allergens, M. paratuberculosis culture filtrates have been purified and fractionated to obtain a more specific and potent product for intradermic testing. Many products have been prepared but very few show promise.

In the development of serological tests, complement-fixation and hemagglutination reactions have been tried. Various types of antigens have been used in the complement-fixation test. It has been found that the complement-fixation test is sometimes positive in animals that are not infected with acid-fast organisms. However, it has an advantage in that sera from an animal in advanced stages of the disease may show a positive titer even though the animal does not react to intradermic johnin.

A modification of the Middlebrook-Dubos hemagglutination test has been used for diagnosing Johne's disease. This test is conducted by sensitizing sheep erythrocytes with johnin PPD. 1/ The sensitized erythrocytes are mixed with sera from suspected animals and observed for evidence of agglutination. The test may show positive results in animals infected with tuberculosis, which of course is a disadvantage. The hemagglutination test can be further modified by the addition of complement and read as a hemolytic test.

At present these diagnostic tests are being evaluated, and I'm going to discuss the results obtained in one infected herd consisting of 180 animals. This herd has been under observation for 2 years. During this period it was tested six times at regular intervals with intradermic johnin; blood was drawn each time for serological tests. The owner sells animals for slaughter only, and intestinal specimens are obtained from each animal slaughtered. Through this procedure the accuracy of diagnostic tests is studied and compared with post-mortem microscopic findings.

The premises leave much to be desired in the way of management practices. As an example, baby calves are permitted to suck old nurse cows and are kept in sheds where the sun never shines. On one occasion two nurse cows that were scouring were each nursing two calves in a shed that was seldom cleaned. Both of these cows were found to have typical small acid-fast bacilli in their intestinal tract when they were slaughtered.

To date, six intradermic tests, six hemagglutination tests, six hemagglutination tests modified by the addition of complement, and three complement-fixation tests have been conducted on this herd.

1/ The precipitated product or purified protein derivative.

A study of the intradermic reactors has disclosed the following information: (1) Of 21 animals that reacted before they reached 1 year of age, 18 (85 percent) lost their sensitivity to johnin within 15 months after the first test; (2) of 31 animals that reacted as adults, 16 (52 percent) also lost their sensitivity within 15 months. (Several reacting animals that were slaughtered in less than 15 months are not included); (3) during the 2-year period, 40 calves were tested at less than 9 months of age and 29 (72 percent) reacted to the intradermic test; (4) of 72 animals from 1 to 15 months of age, 52 (70 percent) reacted to the intradermic test one or more times. Of the 52 reactors referred to, 24 had 2 or more tests after becoming positive; of these 24, 18 (75 percent) did not react on the last two tests. To date, three of the remaining six continued to react. Of 10 animals showing clinical symptoms of Johne's disease since the study started, 7 were reactors to the intradermic johnin test as adults; none of the animals that reacted at less than 1 year of age have shown clinical symptoms to date.

A total of 34 culled animals have been examined post mortem. Nineteen were reactors and 15 were negative to the intradermic test. Small acid-fast bacilli were demonstrated in the intestinal tract of 10 (56 percent) of the reactors and 5 (33 percent) of the nonreactors.

Twenty of the 34 culled animals had positive or suspicious serum titers to the hemagglutination test. Small acid-fast bacilli were found in 9 (45 percent) of these 20, and in 6 (43 percent) of the 14 showing no hemagglutination titer.

Thirty-two of the 34 animals showed positive or suspicious serum titers to the hemolytic modification of the hemagglutination test. Small acid-fast bacilli were found in 14 (44 percent) of these 32, and in 1 (50 percent) of the 2 showing no titer.

Twenty-seven of the 34 had positive or suspicious titers to the complement-fixation test. Small acid-fast bacilli were found in 13 (48 percent) of these 27, and in 1 of the 5 (20 percent) showing no titer. Two had not been tested with a complement-fixation test.

From the results, it appears that the intradermic test is the best single test for Johne's disease, since bacilli were found on post-mortem examination in the intestines of 56 percent of the reacting animals and, at the same time, that the removal of all intradermic reactors would have eliminated only about one-sixth of the herd. However, the test leaves much to be desired, since bacilli were also found in the intestines of five nonreactors, three of which were showing clinical symptoms of the disease. Therefore, if the owner had eliminated all reactors as well as those that showed clinical symptoms, at least two infected animals would have remained in the herd; and since only one-fifth of the herd was necropsied there were probably others.

It is unusual to obtain hemagglutination titers higher than 1:16 in sera from normal cattle. It has not been determined why so many cattle in this herd showed titers well above that level but have not developed clinical symptoms of Johne's disease. Most of these cattle spend their normal productive life in the herd without developing clinical evidence of the disease. Possibly some of them may develop an immunity and have antibodies against Johne's disease.

Products which have shown marked therapeutic effect on tuberculosis and leprosy have been tried for the treatment of Johne's disease. Streptomycin, isonicotinic acid hydrozide, and 4:4' diamino diphenyl sulfone have each been tried on a limited number of animals showing clinical evidence of the disease. None of these products has cured an animal, although temporary improvement was noted in several instances.

Immunization against Johne's disease is being studied in a number of countries. The vaccine is usually made by suspending M. paratuberculosis in an excipient that is not absorbed, such as mineral oil. A fibrocaseous nodule is formed at the site of injection. Vaccination experiments conducted on thousands of sheep in Iceland indicated that marked protection against Johne's disease was established. The vaccine may cause vaccinated animals to react to the tuberculin test, which is a serious drawback to its use in cattle. Since cross-reaction to tuberculin is not an important factor in sheep, we have a limited field trial of the vaccine under way in the United States in an infected flock of sheep.

Sheep in many other parts of the world have also been found infected with Johne's disease. Loss of condition in sheep is generally attributed to parasitism, and many cases of Johne's disease in sheep may be overlooked.

The disease causes sheep to lose condition over a period of time as it does cattle, and, in addition, the wool may be become loose; however, diarrhea is not as marked. The bacillus causing the disease in sheep can be demonstrated in smears or histological sections from the intestinal tract as in cattle. Infected sheep also react to all the diagnostic tests previously described. It has been found to be very difficult to control the disease in this species. In some instances the entire flock has been slaughtered and after a year's time the premises stocked with sheep from a clean flock.

When Johne's disease is clinically diagnosed in a single animal, all the animals in the herd should be tested with intradermic johnin. If the number of reactors is only a small percentage of the herd, they should be removed at once and slaughtered. After the animals have been removed, the premises should be disinfected by the same procedure used in disinfecting a premises from which tuberculin reactors have been removed. All manure and a thin layer of topsoil should be removed from lots used by infected animals, and lots should be arranged in a manner to allow direct exposure to sunlight at some period of the day.

If a large number of reactors is disclosed, the owner may not wish to assume the heavy loss that would result from immediate slaughter. He should be advised to eliminate any animals showing clinical evidence of the disease at once, and to dispose of the remaining reactors by slaughter as rapidly as he can raise young animals for replacement. This procedure of course involves a risk, as reacting animals may be spreading the disease. Breeding stock should not be sold from a herd in which reactors are retained. Even when all reactors are removed from the herd, it should be emphasized to the owner that animals that have not reacted to the test sometimes develop symptoms of the disease, and that he should therefore keep the entire herd under close observation.

The key to the successful control program is to prevent susceptible animals from ingesting infective droppings along with feed and water. Since calves are easily infected, they should be removed from their dams within 12 hours after birth and placed in separate quarters. Attendants caring for these calves should disinfect and clean their footwear each time they enter the quarters. The calf rearing quarters should be provided with separate cleaning and feeding equipment; such equipment should never be exchanged with equipment used for mature animals. Feeding and watering equipment for all cattle should be constructed so as to prevent fecal contamination of the contents. Equipment used for cleaning manure from stables should never used or stored in feed rooms or feed alleys; attendants should change or disinfect footwear before proceeding from areas contaminated with manure to feed rooms.

Suitable disinfectants in footbaths should be placed at the entrances of feed alleys and feed rooms, and attendants should be instructed to use these facilities. Johnin tests should be conducted on all animals in the herd at 6-month intervals. If negative results are obtained on three successive tests and no clinical symptoms of the disease observed in the meantime, the herd may be considered clean.

THEORY AND PRINCIPLES OF CLEANING AND DISINFECTION

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Last year at the Tuberculosis Eradication Conference, I presented a paper on the theory and principles of cleaning and disinfection. This paper may be somewhat repetitious. However, repetition of the basic concepts of disinfection and of their practical application is justified many times over.

I do not propose to discuss the manner and means of transmission of tuberculosis in cattle; however, I know that the tubercle bacillus is frequently present in manure. On this well-founded fact, then, the barn and all its parts, including the air, may thus serve as fomites for the transmission of the disease.

After the removal of infected animals the barn thus continues to be an infected area until such time as all manure, dust and other contamination on surfaces have been removed, and the surfaces have been disinfected to destroy any residual organisms that remain after cleaning. Unless the destruction of all the tubercle bacilli has been accomplished, then, the introduction of noninfected animals into the area is an invitation to continuing the disease.

I am aware that the barn environment is only one area of possible transmission, however; to control the disease, we must close every avenue of possible transmission to the best of our ability.

In an ideal disease program, all avenues of infection would be completely removed. In the control of foot and mouth disease, the disease was eliminated in the United States by drastic procedures, wherein all means of continuance or spread was checked. In some countries tuberculosis is being controlled, not only by good hygienic practice but by destruction of every infected herd and the closing of the premises of such herds for 1 year, before restocking with clean animals. Perhaps such a drastic procedure may be needed in this country, but good sanitation practice and good livestock management may be adequate.

We have been interested during the past year in the development of a better disinfectant for the destruction of the tubercle bacillus. Orthophenylphenol, which has acceptance for such a purpose, was selected as the disinfectant agent. To increase the activity of the germicide wetting agents, solublilizing agents and acids were added. Such a product should have increased germicidal activity, better contact with the organism by the deflocculating action of the detergent, and a rapid wetting action of the surfaces to be treated. Such a product, with a marked increased activity, has been developed.

After laboratory testing, a field test was tried. An average Michigan dairy barn was selected where reactors had just been removed. The barn was supposedly prepared for disinfection when we arrived. The disinfectant-detergent was to be applied as a spray, with a pressure of 400 pounds p.s.i.

The barn was not designed for easy cleaning. The cleaning by the owner consisted of removing most of the loose material. The alleyways were covered with a layer of dried manure that was imbedded in the rough surface of a poorly laid concrete floor. The stanchions were covered with manure and grease, and the feed troughs were littered with chaff and dust.

All that was needed for disinfection was a supercompound that could be sprayed over the surfaces so that some magical agent in the compound could seek out each tubercle bacilli, hidden in the thick masses of organic debris, for destruction. Unfortunately the disinfectant-detergent was not a supercompound, nor does such a compound exist that could cope with the mass of manure on the surfaces in this barn.

Although disinfection was impossible, the compound was tried to learn more about the effectiveness of a wetting agent and detergent in the presence of heavy contamination. The compound worked successfully in quickly wetting the dried manure and emulsifying the manure in a foamy mass. The surfaces were rapidly freed of organic matter. The test demonstrated the value of a detergent in a high velocity spray as a means of cleaning; however, such a preparation would be far too expensive to use as a cleaning agent.

To demonstrate the ineffectiveness of the disinfectant-detergent under such adverse conditons, swab samples were taken before and after cleaning more of the manure from a small section of the barn by use of the spray.

Swab count of the floor before treatment showed a total count of 3,830,000, which is probably low owing to the dryness of the manurial surface. After treatment the floor yielded a count of 1,320,000. In an area where wetted manure still covered the surface, a count of 1,220,000 was obtained from swab sample taken just below the surface of the manure. The surface of a dusty beam yielded a count of 600,000; after spraying the count was 11,700. These data show the inadequacy of disinfection of badly contaminated areas.

Organic matter may act as a protective agent for the organism against the germicidal activity of a disinfectant used alone. This is especially true when the organism is imbedded in a layer of organic matter, such as would be the case for an organism in dried manure. Even though the disinfectant could penetrate through the organic matter barrier, the amount still active might be far too small to exert bactericidal activity. The organic matter reacts with the disinfectant by combining and forming an inert compound, or it may merely mechanically absorb the compound. In either case the disinfectant is rendered permanently or temporarily inactive.

When a detergent is used in combination with a disinfectant, better results may occur in the presence of organic matter, because the detergent causes a deflocculation of the organic matter, and suspends the resulting particles. Thus, the organisms are freed so that the disinfectant may attack directly. However, if the organic matter load is high, then the detergent is used up and complete deflocculation and suspension fail to occur. Also, the suspended particles may absorb the disinfectant.

For a detergent-disinfectant to function, the organic matter should be removed, so that all surfaces appear clean. When a detergent-disinfectant is applied to such a surface, the detergent tends to aid in wetting the surface, deflocculating minute particles of organic matter, and disinfection occurs in greater degree than might be obtained with a disinfectant alone, such as sodium orthophenylphenate.

The infuence of the wetting agent as an adjuvant to orthophenylphenol is demonstrated by the relative bactericidal activity demonstrated by the phenol coefficients. When a phenol coefficient of 15 was obtained with Staphylococcus aureus with sodium orthophenylphenate, the experi-

mental detergent-germicide containing orthophenylphenol gave an equivalent phenol coefficient of 45. Thus, the activity of the orthophenylphenol was increased threefold. It must be remembered that the presence of organic matter would lessen the germicidal activity of the orthophenylphenol to the same degree in both preparations. The detergent-germicide with a third of orthophenylphenol content would be more effective on a properly cleaned surface than would sodium orthophenylphenate alone. If the detergent-germicide were used on dirty surfaces, a cleaning action would result from the action of the detergent and a lessening of bacterial population would result; but disinfection would not result unless the treatment were continued until the surfaces were free of soil. It would be far more economical to first apply a good detergent of less cost to remove the soil, then apply the detergent-germicide to the cleaned surface to destroy any stray bacteria that were not removed by the mechanical cleaning process.

When a disinfectant or detergent-germicide is placed in the hands of fieldmen, many things happen in the preparation of the disinfectant solution and its application that may not be conducive to good disinfection practice. Some of the adverse happenings may be inherent in the disinfectant, but most are due to the operator.

To find what may happen, a small plastic bottle (20 ml. capacity) and a sheet of directions was sent out to a randomly selected group of fieldmen employed by the Michigan Artificial Breeders Association. The instruction sheet requested each worker to prepare the disinfection solution on one of the farms visited, but before using it he was to fill the bottle with the freshly prepared solution for mailing to our laboratories. Each of the samples was tested for germicidal activity at the laboratory by checking the degree and rate of kill of Escherichia coli.

Out of 105 samples received, 21, or 20 percent, failed to show slight, if any, germicidal activity. Eighty-one of the samples were quaternary ammonium compounds; of these 15, or 18.5 percent, were ineffective. Although in some cases the operator used too little disinfectant in the preparation of the solution, many of the solutions had been inactivated by the use of incompatible waters. Of 15 disinfectants listed other than quaternary ammonium compounds, 7, or 46 percent, were ineffective. Four samples contained a pine oil disinfectant, and three, or 75 percent, were ineffective.

On the assumption that the above survey is a fair cross section of this group of fieldmen, one out of every five is failing to disinfect equipment at each farm. These workers failing to disinfect equipment between farms could act as mechanical carriers in the spread of disease.

The experimental detergent-germicide containing orthophenylphenol that is unaffected by hard waters was sent out to the same workers used in the previous survey. The directions for collecting samples were the same. Of the 78 samples received to date, 7, or 9 percent, failed to show germicidal activity. The failure to show germicidal activity in seven samples may have been due to improper dosage; but in some instances,

solution had an odor of manure, and was deeply colored, indicating a misuse of the disinfectant solution before sampling. Fresh solutions were requested for test purposes.

Although a better disinfectant was supplied in these experiments, the solutions that were properly prepared were likely improperly used. The data do not show the extent of misuse, but observations indicate that little germicidal activity occurs because of misuse of the disinfection solution.

In the case of the fieldmen in the experiments cited, most of these men have had little or no training involving the use of disinfectants and their proper usage. Unfortaunately, many professional people, who should understand the limitations of disinfectants, attempt disinfection in the presence of gross contamination; many of these people believe that disinfection, has been accomplished.

Cleaning and disinfection is important in preventing the spread of disease by fomites. To protect the animal from infection from its environment, good sanitation practice is necessary. Care should be exercised to treat potentially contaminated areas intelligently, by use of good cleaners and sanitizers. A properly sanitized surface is a safe surface.

VII. PROFIT FROM WORLD WIDE KNOWLEDGE

TUBERCULOSIS IN ZOO ANIMALS

Charles R. Schroeder, Director, San Diego Zoological Gardens, San Diego, Calif.

In 1938 at the New York Zoological Park I examined a spider monkey (Ateles ater) with a groin suppurating abscess. On examination it was apparent that this was of long standing involving a superficial inguinal lymph node. The animal was prepared for simple surgery. The greatly enlarged node was excised and skin closed. All would have been well, but on laboratory examination human tubercle bacilli were shown in the exudate. The animal was destroyed and demonstrated generalized disseminated tuberculosis.

In the thirties one animal dealer regularly sold rhesus monkeys, not acceptable to laboratories, as household pets. On three occasions I personally had monks shown to me with "sores that don't heal," usually proving to be cervical or inguinal scroflula.

In a wild animal show during the New York World's Fair, an exciting monkey island on the midway was the boardinghouse for a dealer's rhesus monkeys. By actual sampling it was shown that the frequency of human-acquired tuberculosis and dysentery was high in this group. Dried feces were wafted on the breeze to the food in hand of the visiting public.

In 1941 a performing chimpanzee, privately owned, was submitted for diagnosis. Eye grounds examined by a neurosurgeon suggested brain tumor. The animal was prepared for surgery. A large tumor mass was removed from the occiput which proved to an infectious tuberculous granuloma involving all meninges, as well.

Traffic in monkeys, mainly Macaca mulatta (rhesus), starting in the late twenties grew rapidly through the thirties. Political and religious problems arose causing seasonal embargoes on their importation. For obvious reasons Mohammedans were used to capture the monks, Hindus to maintain them. Collecting depots at shipping points were notably unsanitary and maintained in communities where the incidence of human tuberculosis was high. Shipments were made by boat, to economize on transportation. Monks were greatly overcrowded in crates, poorly withstood the transocean voyage; many died in crates and because of the escape problem the dead were not moved from the crates. Many deaths were due to TB and the carcasses were actually consumed by the other monks or directly contaminated the food, a survival diet, thrown into the crates. The crates were not actually cleaned until the monks reached destination, usually the port of New York. A safe guess, made with the support of a sampling program both at a dealer's warehouse and a large laboratory, is that 50 percent of these animals had active tuberculosis.

Today, although many rhesus are still being used, the majority are M. cynomolgus from the Philippines. Although tuberculosis is still a problem it is of minor importance in the overall health picture of these monks, because of greatly improved collecting techniques, transit handling procedures, rapid air shipment, better diet, and avoidance of crowding, but unfortunately all at greatly increased final selling price--from $6 to $40 in a 25-year period.

Today, of the estimated 25,000 monks imported monthly from both India and the Philippines, we find little or no TB in those from the Philippine source, and only about 1 percent in the Indian source.

Unlike host specific, helminthic and ectoparasitic disease, certain blood protozoan diseases, pasteurellosis, salmonellosis, and viral diseases, there is little evidence to show that tuberculosis occurs in wild animals in their native habitats. There is ample evidence, however, to show that most exotic species are susceptible to infection from Mycobacterium tuberculosis. Human, bovine, avian, and reptillian types have all been isolated from captive wild animals, and the occurrence has been widespread. Many acid-fast organisms have been isolated from fish, reptiles, and amphibians. In each instance the source of infection has usually been apparent.

In a survey made within the last 2 weeks involving 12 zoological parks, by personal communication, it becomes apparent that tuberculosis is still with us, but no longer a major problem.

To give an idea of some of the odd hosts reported to have died with tuberculosis in the last few years, I list here: Many reptiles, including Mata mata turtles; giraffes; muntjac; both American and Asiatic tapirs; capybaras; otter; elands; rhino; bison; many species of deer, including elk and moose; many primates, including orangs, chimpanzees, and gibbons.

Alaskan reindeer have high frequency, but many Scandinavian reindeer have been imported by zoos within the last year or two and I know of no report of tuberculosis in these animals.

TB in birds seems still to be relatively common, including frequent occurrence in most captive gallinaceous fowl-peafowl, wild turkey, pheasant, etc.

Reliable morbidity and mortality data on TB in captive wild animals are lacking, but greater attention is now being given to the need for post-mortem examination by competent pathologists leading to compilation and publication of findings. With the meager material at hand, it is not possible to present significant data concerning species susceptibility. The average zoo has two to three hundred species of animals, and the largest zoos one thousand. What appears to be high susceptibility in a particular species in one zoo may be low in another.

Whereas previously it was oonsidered that all primates had unusually high susceptibility to TB, the San Diego experience indicates that this is not so. Routine and careful post-mortem examination gives no evidence that the San Diego Zoo, which includes 58 species of primates, has had a single case of acquired tuberculosis in mammals. Fortunately, only a few infected animals and birds have been brought in from other zoos and dealers, discovered post-mortem shortly after they were received.

If I may be permitted the luxury of theorizing, I propose that animals kept in the out-of-doors, even though having contact with infected humans, do not acquire tuberculosis because of the factor of great air dilution. The prospective host does not receive an infective dose large enough to overcome his resistance. In our zoo, with a population of almost four thousand animals, representing a thousand species, we have demonstrated tuberculosis only in a newly acquired wading bird and a few reptiles this year. By contrast, concerning animals in zoos requiring heat and indoor housing where public, keepers, and animals live in a confined and limited air volume, the incidence of tuberculosis is much higher. Where primates are completely excluded from the public by glass partitions the frequency has been very low in recent years.

Perhaps of greatest importance is the need for the prompt and frequent application of a practical and foolproof diagnostic test which can be applied to all newly acquired animals. The test presently used in primates is the administration of 1 mg. of PPD, in a 0.1-cc. dose administered subcutaneously just above the margin of the upper eyelid, with a 1-cc. tuberculin syringe and a 3/8-inch, 27-gauge stainless needle. The test is read between 24 and 72 hours. In those zoos where tuberculosis

has been a problem, or where the incoming animal has been acquired from a zoo known to have experienced tuberculosis, the standard intradermal test, using O. T., as applied to cattle might well be applied to all newly acquired cloven hoofed animals.

Other than having the dubious pleasure of looking at people, zoo animals have a rather drab existence. We place them in homes built to our specifications, prepare their diets--and we hope what's good for them; lights go out when the sun goes down; they are up in the early morning, have rather well regulated exercise; we more or less control the air they breathe and permit little or no dissipation. The finger can be pointed directly at management for most illnesses and deaths. I submit that a zoo is an ideal unit for the study of epidemiology of a population where all usual freedoms have been removed.

Most zoos today are concerned about the personal health of all those who have contact with animals. Medical examinations, with chest pictures, have become routine. Most food is suitable for your own table. The obvious great reduction in tuberculosis is undoubtedly due to these many management improvements and the removal of many of the stresses and strains previously endured.

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TUBERCULOSIS ERADICATION IN GREAT BRITAIN

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In describing the work that has been done to eradicate bovine tuberculosis from Great Britain, it must be understood that the methods followed and the tests used have been devised to meet the prevailing conditions and that they are not necessarily applicable elsewhere.

The incidence of disease in the country was comparatively high. It was estimated in 1931 that 40 percent of cows would react to the tuberculin test. In 1938 a tuberculin survey was carried out when 12,300 self-contained herds were tested, comprising 364,286 cattle, and 13 percent of the animals of all ages reacted. It must be emphasized that this survey had, as part of its purpose, encouragement to owners of self-contained herds to proceed with eradication at the early stages of the campaign, and that the incidence in these herds is lower as compared with dairy herds for which replacement cattle are purchased. Infection was not equally distributed. In England 14.1 percent of the animals reacted, while the

figure for Wales was 4.1 and for Scotland 23.1. Large herds had the highest number of reactors.

2,432 herds with an average number of animals of 50.4 had over 10 percent reactors
2,897 herds with an average number of animals of 39.6 had less than 10 percent reactors
6,971 herds with an average number of animals of 18.24 had no reactors.

While the survey was not by any means complete, it was sufficient to show that the task of eradication was to be a heavy one. The survey confirmed also that the incidence of infection was highest where animals were managed intensively, for example in the milk shed of the large industrial areas. The incidence too was low in beef type herds and in less heavily stocked areas. Management and methods of husbandry obviously played an important part in the spread of the infection. In most of the dairy herds which breed their own stock, the young cattle are maintained apart from the adults until they are ready to calve down for the first time, when they are added to the milking herd. It has been a common experience that the milking cows contained a high percentage of reacting animals, particularly if housed under poor conditions with inadequate lighting and ventilation. In contrast, the young stock was remarkably free of infection; although occasionally a particular batch of heifers might be infected as a result of being reared on the herd milk at a time when a cow with a tuberculous udder was present. The fact that the young stock was remarkably clear of infection led to the development of methods of eradication, which will be described later.

Since 1925 there has been authority to slaughter cattle clinically affected with tuberculosis, or giving tuberculous milk, or excreting or discharging infective material. There were 23,716 animals slaughtered under this Order in 1936; in 1958 the figure had fallen to 350.

In 1935 the Attested Herds Scheme was introduced in an effort to encourage eradication. Under this scheme, which has been amended from time to time to meet changing conditions, the farmer is required to produce evidence that his herd has been tested at his own expense on two successive occasions and that at neither test were any reactors disclosed. When this evidence is available, an official test by a whole-time member of the Ministry of Agriculture and Fisheries' veterinary staff is carried out, and if no reactors are found, the herd becomes an attested herd. If reactors are found at this official test, they are disposed of, disinfection is carried out, and a test at about 60 days after completion of disinfection is made. Such short-interval tests are repeated until the herd is cleared of infection, when it becomes an attested herd and is then subject to test in 6 months, and eventually at intervals of 12 months so long as no reactors are found. Rules were devised to protect attested herds from reinfection, and they have remained unchanged. They include the prohibition of feeding milk to calves unless it has come from another attested herd. There are also provisions to prevent contact with animals on neighboring farms, and this may involve the erection of double fencing.

All animals moved to the herd require to be moved on the authority of a permit. Those from another attested herd or from a sale authorized for attested animals move into the herd without condition; but from other sources the animals require to be isolated for a period up to 60 days before being added to the herd following a satisfactory retest. In order to encourage owners to clear their herds, every effort has been made to advertise the advantages of eradication and to show how much healthier is a herd which is free of tuberculosis. However, it has been found that some form of direct incentive has been necessary to encourage owners to participate. This, along with the fact that the animals from attested herds have demanded higher prices, has done a great deal towards the progress of eradication. At present the bonus payable to attested herd owners is either 2d per gallon of milk sold for 4 years and 1d per gallon for 2 years following attestation or £2 per head of animals in the herd for 4 years and £1 per head for 2 years. In the peak year over £10 million was paid as bonus.

All tests of attested herds are done at public expense, and most of the tests are done by local veterinary inspectors who are veterinary surgeons in private practice employed on a part-time basis. Last year L. V. I.'s were paid £1.7 million in fees, which includes a comparatively small sum for work other than tuberculin testing.

It will be noted that the main burden of eradication is placed upon the farmer himself in that he is required to produce evidence of two successive clear tests. Veterinary surgeons have devised various methods of eliminating infection in herds. Where there are only a few reactors it is very simple to remove them, carry out disinfection, and make short-interval tests until the disease is eliminated. In more heavily infected herds it has been necessary sometimes to segregate reactors from the nonreactors and to eliminate infection more gradually. In herds where there is very heavy infection, it has been found advisable to remove all the animals in the infected group and, after careful disinfection, to replace them with animals from attested sources. In herds where this practice has been followed, the subsequent history is very satisfactory. In such herds, if the young stock has very few reactors, they can be retained and serve to maintain desirable blood lines, particularly in valuable herds.

In the first 2 years only 414 herds had become attested, and progress was, of course, slow during the war when the activity under the schemes was limited. Seven percent of the cattle were in attested herds at the end of 1944; 19 percent in 1949; 76 percent in 1957; 85 percent in 1958; and at the end of June this year 90 percent of our cattle were in attested herds or areas.

In the course of the years it had become obvious that the tuberculin originally in use was not sufficiently potent to discover all cases of bovine type tuberculosis. With this in mind and after trial tests it was decided to produce tuberculins on the same method as was followed at that time by the Bureau of Animal Industry. When this more potent tuberculin was introduced it disclosed an alarming percentage of reactors in

herds which had previously been judged to be free of infection. Investigation of this problem revealed that practically all of these reactors were sensitized to tuberculin by organisms other than bovine-type tuberculosis. As a result, a comparative test, using mammalian and avian tuberculins, was devised in order to differentiate between animals sensitized by bovine-type infection and by other infections. It was decided that the essential feature was to remove the animals with bovine-type infection and to ignore those sensitized on account of avian tuberculosis or skin tuberculosis, because as a general rule these infections do not spread from one bovine animal to another. A number of animals are also sensitized as a result of infection with M. johnei, but not all animals so infected react to tuberculin. It is therefore considered that the problem of Johne's disease is an entirely separate one and that the position in regard to this disease is not affected by the retention of animals which may be sensitive to tuberculin because of it.

Eventually P.P.D (purified protein derivative) tuberculins came to be used, and we now know that each successive batch of tuberculin is virtually identical.

The single intradermal comparative test is used on the basis of a herd test. It is not a test which can be recommended for individual animals of unknown history. In the herd test a general assessment of the reactions is made and if it is clear that sensitivity is arising from causes other than bovine tuberculosis, one system of interpretation is followed. A much stricter interpretation is applied if the herd is in the process of eliminating infection or if it is obvious, as evidenced by the reactions in one or more animals, that bovine-type infection is present. The single intradermal comparative test has its greatest value for maintaining freedom from bovine-type infection in herds from which the disease has been eliminated. It is a test which makes it possible to eliminate only those animals which are infected with bovine-type tuberculosis, which is essentially the disease of cattle, and not requiring to eliminate all animals which for one reason or another are sensitive to tuberculin. Obviously this test is preferable in Great Britain, for in our attested herds and areas we have approximately 17 percent of animals sensitive to mammalian tuberculin and 35 percent sensitive to avian tuberculin. These are not considered to be affected with bovine-type tuberculosis.

Area Eradication

When we had reached the stage when some 20 percent of cattle were in attested herds, an area plan of eradication was devised. It follows three stages. In the first stage free tuberculin tests are carried out on application by owners of herds which have not yet been cleared of infection. This free testing is granted for a period of 2 years, and at the end of this time the area is declared an eradication area. In an eradication area all herds not having had two clear tests are tested compulsorily and reactors slaughtered with compensation to the owners. These herds do not qualify for bonuses under the scheme. When all the herds requiring compulsory tests have been tested twice, with reactors removed and disinfection completed, the area is declared an attested area.

It is only in eradication and attested areas that reacting animals are slaughtered, except that owners of attested herds which have been established for over 4 years may choose to have reactors slaughtered with compensation rather than to dispose of them privately. Compensation is the full value of the animal, subject to a limit of £100.

In attested areas there is free movement of animals within the area but the area is protected against the introduction of infection in the same way as an individual attested herd--permits are required for the movement of animals into the area.

In attested herds the normal interval between tests is 1 year. It is intended to increase this interval in attested areas and, so far, the interval has been increased to 2 years in the earliest established areas. It is our intention always to test all the animals in a newly established attested area in a year to a year and a half from the declaration of the area. Only if the percentage of reactors is very low and if the number of infected herds is also low, do we increase the interval between tests. Our intention is to maintain a maximum of 3 years' interval between tests, at least until we are satisfied that the reactor rate has dropped to an extremely low figure and that the other methods of ascertaining infection are working satisfactorily. These methods will be the disclosure of infection in abattoirs and knackeries and reports from public health authorities in regard to tests of bulk milk, and so forth. Certain herds will be tested more frequently in accordance with local knowledge of the veterinary staff. Any herd in which a reactor is found will be tested at 60-day intervals until free of infection and again at 6 months; only then will the normal interval of the locality be restored.

In 1941 the percentage of reactors disclosed in attested herds was 0.41; in 1946 it was 0.46. Since then the figure has fallen steadily, to 0.14 in 1958. Comparable figures in the areas are--

1953	0.26
1954	0.2
1955	0.18
1956	0.11
1957	0.09
1958	0.11

It is evident, therefore, that the area procedure is resulting in a considerable reduction in the infection rate.

It is perhaps of interest to note that very few reports are received of infection in attested herds or areas from abattoirs and knackeries. The results of post-mortem examination of reactors has been as follows: 27,315 reactors showed that 72.25 percent of them had visible lesions of tuberculosis at a straightforward abattoir post-mortem examination. If it be accepted that animals slaughtered as reactors are themselves affected, if they are herd companions of reactors showing visible lesions, then the figure rises to 83.28.

It is anticipated that in October 1960 the whole of Great Britain will be one large attested area. This obviously poses a number of very important questions connected with the maintenance of freedom from bovine tuberculosis. The situation in already established attested areas leads one to the conclusion that it is possible to eliminate bovine-type infection. The big reservoir of the organism is obviously cattle and the reduction in the infection rate will eliminate most of the sources of reinfection to tubercle-free herds. It is true that there are occasionally animals which, having a quiescent lesion at the time of a test, fail to react and later the lesion breaks down and the animal becomes infective. Thus a previously tubercle-free herd may show a high incidence of reactors. It is because of this particular risk, and experience of the phenomenon, that it is considered advisable to ensure that all animals in an area are tested at a regular comparatively short interval, at any rate for some years. Other sources of reinfection may be other types of farm animals. We always submit goats that are in contact with cattle to the tuberculin test and eliminate reactors. In our experience other animals have not been an important source of reinfection and it has been found, as in other countries, that the infection rate among other farm animals, notably among pigs, falls steadily in parallel with the fall of infection rate in cattle. The generation of these farm animals is comparatively short. There is, however, one reservoir of infection which must be carefully guarded against. Bovine-type organisms may infect man and may, indeed, produce pulmonary tuberculosis. Since man has a sufficiently long life to bridge the gap between the time when there was a heavy weight of infection amongst our cattle and the present comparative freedom in our herds, cattle may become infected from workers on the farm from time to time. This has not been a common occurrence among our herds, but it has occurred and would become a much more important element if the interval between tests in our attested areas was unduly lengthened, since infection would have a longer opportunity to spread in and from the herd.

After the whole country becomes an attested area we consider it necessary for the maintenance of freedom from infection to organize tests of all herds at regular intervals. The interval between tests should not increase beyond 3 years until experience shows it to be reasonably safe. Other means of disclosing infection must be developed, e.g. reporting from abattoirs and knackeries. Infection disclosed by any such means must be followed up and the herds involved must be tested. There must be careful tracing of animals which may have brought infection to a herd in which reactors are found, and similarly animals moved from such herds must be traced to their new herds and tested there. Close liaison with the medical authorities will be necessary to deal with the possible risk of reinfection of herds of cattle from human sources. In all cases where reactors are found strenuous efforts must be made to discover the source of infection. Efforts to improve and sharpen the tuberculin test should continue, for it is evident that, paradoxically, a more accurate test is required as complete eradication of bovine-type infection approaches.

TUBERCULOSIS ERADICATION IN CANADA

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With the end of our bovine tuberculosis eradication program now in sight, there is a tendency to overlook the intervening years of accomplishment and, also, to forget the fact that the job has not yet been quite completed. Over the years, valuable information has been accumulated, and, in presenting this paper, I should like to review the progress that has been made and describe how our Tuberculosis Eradication Program is functioning in Canada today.

Although testing was carried out as early as 1900 in Canada, the first herd plan, known as the Supervised Herd Plan, was embarked upon in 1908. This was a voluntary herd plan and was subsequently abandoned.

In 1919, the Accredited Herd Plan was adopted. Today, there are approximately 10,000 herds and some 450,000 cattle under this program. Since the inception of the Accredited Herd Plan, 11 million tests have been undertaken and 90,104 reactors uncovered.

The testing of cattle on an area basis was commenced in 1923 under the Restricted Area Plan. This plan replaced the earlier voluntary herd programs and, today, all of Canada has been declared a Restricted Area.

Since the introduction of the Accredited Herd Plan and the Restricted Area Plan, we have reduced the infection rate from approximately 3.28 percent to an all-time low of 0.14 percent, as recorded in the last fiscal year. Since the inception of these testing programs, a total of over 48 million tests have been conducted and 565,545 reactors removed. The total compensation paid to livestock owners since these programs were introduced has amounted to $21 million ($20,758,909).

As a result of these testing programs, the average incidence of tuberculosis infection found on post mortem in Inspected Establishments has been progressively reduced. This is reflected in the percentage of carcasses which are condemned on account of generalized tuberculosis. In 1928-29, the rate was 0.44 percent, and in the last fiscal year recorded (1957-58), it had been reduced to 0.01 percent, a reduction of approximately 44 times.

We, in Canada, are proud of these accomplishments, and much of the credit must be given to our predecessors who introduced and developed the program, sometimes in the face of considerable public opposition.

Forty years is a long time to devote to any program, and we might well ask ourselves why it has taken so long and why isolated foci of infection still remain in a few herds.

During the early days of this eradication program, our officers acquired a tremendous amount of valuable information in the application

and interpretation of the tuberculin test, and much progress was made toward eradication. However, within the past decade, many of our experienced field officers and practitioners have retired or are no longer with us. The younger veterinarians who have replaced them have not had the experience which their predecessors possessed. In many instances, they have seen very few tuberculin reactions and, in some cases, have been disappointed when reactors, which they did uncover, proved to have no visible lesions. This, I believe, has led to some complacency on the part of field officers and has fostered the attitude that tuberculosis has been, to all intents and purposes, eradicated, and that what they are encountering periodically can be attributed to a nonspecific reaction. This attitude is dangerous, since we all know that it was much easier to uncover the first one hundred thousand reactors than it would be to uncover the last thousand.

The significance of this all-time low level of infection, 0.14 percent, is fully appreciated when translated into the number of tests required to uncover a reactor. Today, at this level of infection, we have to test 715 cattle in order to uncover one reactor. In the early years of the program, one reactor was uncovered, on the average, for every 30 cattle tested and, in heavily infected areas where the rate of infection ran as high as 20 percent, this figure of 30 cattle to be tested would be considerably lower.

We believe that, if our goal of total eradication is to be achieved, we must place greater emphasis on the proper training of our field inspectors. This is no easy task. Years ago, we could provide excellent on-the-job training with the assurance that we would be able to demonstrate tuberculin reactions within a very short period. These conditions no longer prevail.

To offset this, arrangements have been made to have animals sensitized with killed tuberculosis organisms, and, in this way, demonstrate tubercular reactions to students at our veterinary colleges. To a point, this procedure has served a useful purpose; unfortunately, some of the reactions given by these sensitized animals are rather large and do not represent the typical reactions which one encounters in a herd where only a few animals are affected.

One can assume that no one will overlook a badly infected herd. The danger of missing reactions, we believe, is in herds where only one or two animals may show slight reactions. We have also embarked on a further training program in the case of practitioners employed as casual, or part-time, veterinarians. These men are required to undergo an 8-day training period with a full-time, salaried officer. During this period, great emphasis is placed on the application of the test. The various techniques of injection are explained, and the veterinarian is not permitted to undertake testing as a casual veterinarian until he has mastered these techniques to the satisfaction of the training officer. It is felt that if good intradermal injections are made, then it should not be difficult to interpret the test. During this training period, the practitioner

receives full pay at the rate of $30 per day. This procedure eliminates any objections the practitioner might have to accepting a period of training.

Even though a period of training and instruction has been provided for both full-time and casually employed veterinarians, we believe it necessary to supervise all testing in Area Programs. This is most important since we find officers are inclined to develop their own idiosyncrasies and standards in the application and interpretation of the test. It is only through constant supervision that a uniform application and interpretation of the test may be obtained and possible foci of infection not be overlooked.

Testing in the field under range conditions can be arduous; and, without restraint, proper injections and interpretations cannot be made. Tuberculin testing in modern dairy barns, with ample help and individual tie-stanchions, is quite different from testing at community pastures or on the open range. (It is in the Canadian range country where the last few thousand cattle to be tested are located.) Under these latter conditions, corrals and testing chutes, if not available, have to be built. Surprisingly, few ranches have testing chutes and, peculiarly too, many ranchers are not too familiar with their construction. Arranging with owners for these facilities takes time, but without these chutes the efficiency of the test is lessened. Our Division does supply mobile chutes which help overcome, to a degree, this problem.

In addition to devoting our limited manpower to completing the testing, we are emphasizing two particular operations in our testing program. One of these involves the tracing of nonreactor cattle which have lesions of tuberculosis on post-mortem inspection. We feel it is essential in our eradication policy to trace these animals. This is possible through our ear-tag distribution system. These tags are purchased by the Federal Government and are coded by the districts to which they are issued. Within each district, tags are distributed to subdistricts and, again, only certain series are assigned to each subdistrict. Individual inspectors record the ear-tag numbers that they use consecutively. The unused tags are turned into the subdistrict office. This system has enabled us to trace the source of nonreactor cattle which have shown lesions on slaughter regardless of where they originated.

The other operation we stress is tracing the movements from any herd in which we find reactors. All sales and purchases for the past 2 years are traced. All herds from which reactor animals originate and all animals sold for breeding purposes are submitted to a special test. In this manner, we have uncovered sources of infection by studying the epidemiology of reactor herds.

Under the Animal Contagious Diseases Act, there is provision whereby a veterinary inspector may order animals slaughtered which have been exposed to infection although they do not react to any test, nor show clinical symptoms of a disease. It has been standard procedure to order

the slaughter of calves that are the offspring of herds badly infected with tuberculosis. During the past few years, we have utilized this authority to remove adult animals in badly infected herds.

In carrying out the standard intradermal test, 1/10 of a cc. of tuberculin is injected by using a 24-gauge, 3/16-inch medium bevel needle, midway along the ventral border of the right caudal fold. The site of injection is first cleaned with absorbent cotton, moistened with ether or alcohol. Readings are conducted at the 72d hour, except in problem herds where we often conduct readings at the 72d and 96th hours. All animals injected with tuberculin are ear-tagged at the time of the injection. This applies to all animals in the herd, including calves. Reactors are identified with red ear tags placed in the left ear. This ear tag has the word "reactor" stamped on the upper side.

The Federal Government provides compensation to owners where reactor animals are ordered slaughtered. The maximum compensation for purebred animals is $140 and $70 for grade animals. In addition to this field compensation, the owner receives the salvage value of the carcass from the abattoir. If the carcass is condemned as unfit for food, the Government pays, in addition to the field compensation, the value of the carcass if it had been passed for food.

All tuberculin used in the eradication program is made at the Animal Diseases Research Institute in Hull, P.Q. Before each new batch is released for diagnostic purposes, it is tested under field conditions by the caudal fold method in parallel with the corresponding Provisional Standard Tuberculin on about 300 head of cattle. This, of course, is in addition to the technical tests conducted in the laboratory during its processing.

In Canada, reactors in which gross lesions cannot be demonstrated on post mortem are recorded as "N.V.L.'s"-- no-visible-lesions. Such cases can be attributed to two possible causes. Firstly, under slaughter house conditions it is not always possible to find a very minute lesion. Secondly, a large percentage of reactors showing no lesions on post mortem are due to cross-sensitization. This may be caused by avian or human strains of the tubercle bacillus or by Johne's bacillus. Saprophytic acid-fast bacilli might also produce cross-sensitization.

This has been a brief review of some of the accomplishments in our tuberculosis eradication program in Canada. In the foreseeable future, all cattle in Canada will have been tested. Although there are many detailed operations which have not been covered in this presentation, each and every phase of the eradication program is important, no matter how minor. A great deal of manpower, time, and money has been expended in our Tuberculosis Eradication Program, and, although some problems are still being encountered, we feel that we will eventually eradicate the disease from Canada.

BOVINE TUBERCULOSIS IN GREECE

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In introducing the problems with tuberculosis in Greece it should be stated that during my memory the problems have been similarly serious in New York State and in other concentrated dairy cattle regions of the world.

The seriousness of the disease in the early days in herds in the rural area near Buffalo, N. Y., can be illustrated by two personal experiences.

On a farm where I worked in 1917, there was a high-producing emaciated-grade, blue and white Holstein cow that suddenly developed acute bloat after having chronic tympany for some months; and as she was in extremis was slaughtered for the local market. The foreign laborers in a small city near Buffalo were glad to secure cheap beef. The small butcher of foreign extraction had no qualms about supplying it, and the farmer salvaged something for a carcass which was a typical case of "pearl" disease with multiple tubercles covering the pleura and peritoneum.

The second episode concerned a progressive neighbor near our home farm. He had purchased a large dairy type Holstein foundation cow and a few young cattle from a farm near Rochester in 1906 and built up a fine herd by 1917. It became necessary to have the herd tested by the temperature test in order to sell cattle. In "preparing" for the application of the test my very honest, well-meaning, misinformed breeder friend and neighbor showed me the tuberculin and syringe that some farmer's biological supply house had willingly sold him. He planned to "plug" or desensitize the herd a few days in advance of the application of the temperature tuberculin test by the local practitioner. He suspected that he might lose a few good cows unless he followed this plan of "plugging," which was in use all too widely at that time until the intradermal test was found capable of beating the desensitizing or "plugging" procedure.

The progressive breeder's son, a man about my age, was quite surprised when I, then a veterinary student, explained to him that he could not live with a herd with tuberculosis nor could he sell them as sound just because they could be made to pass the test.

This purebred herd was finally tested in small groups so that there would be some milk cows left from which to have a small income. A few young cattle were all that finally failed to react. The old foundation cow with extreme emaciation, an arched back, and a huge udder that still produced fairly well was, of course, a generalized case of tuberculosis.

With ample education of the owner and the assistance of his veterinarian and with funds for paying indemnity, this neighbor was soon able to develop a tuberculosis-free herd and their descendants have formed a well-known herd in existence today in Ohio. It did require a complete

tearing down of the system of drinking cups and pipes and boiling these in a lye solution before reactors stopped appearing in this original herd that was saturated with tuberculosis.

Tuberculosis in native and crossbred cattle in Greece in 1958 and 1959 was strikingly similar to our own situation in 1917. The American Farm School, a progressive model farm at Saloniki, had been given pure-bred Jersey cattle, and two complete herds were lost with tuberculosis. The barn was then burned to "disinfect" the premises; after a new barn was constructed, a third self-contained herd of fine Guernseys and Jerseys from America was secured and tested and classed as tuberculosis free. All of the visiting American public officials in Saloniki purchase high class brucellosis-and tuberculosis-free pasteurized milk from this small model herd. The management, however, dreaded the tuberculin test so much that they did not apply it, but hoped the disease was being kept out by "isolation."

It was easy for me to find evidence of tuberculosis on clinical examination in many "village" herds in Northern Greece. A peculiar nodular type of fibrosis of the udder was proved at autopsy to be tuberculosis of the udder in one of the first cows which we diagnosed clinically.

In examining cattle for sterility it was possible occasionally to find adhesive peritonitis and perimetritis with characteristic nodules of tuberculosis present, and at the same time, in one cow, a tuberculous udder.

A visit to the city abattoir of Saloniki would have been a wonderful education for a modern veterinary faculty and student body from our American colleges. Extensive generalized tuberculosis was common, and specimens were readily and frequently secured for instruction of students in the Greek Veterinary College at the Aristotelian University of Thessaloniki. Tuberculosis of the vertebrae of swine was also observed.

The application of the tuberculin test to cattle in Greece was done by a very careful injection of the subcaudal fold only while the tail was held well elevated. The purchase of cattle was sometimes preceded by this test with the owner being instructed in reading the test to save the expense of a return trip over bad or no roads. If the cow reacted nothing was done; if she did not react she was accepted by the purchaser. This is to me, essentially, living with tuberculosis.

An opportunity to study the reactions following a test in one village herd of perhaps 100 head owned by approximately 30 different people (village farmers), but assembled in close contact with other cattle and with the village families, showed at the point of injection very frank reactions with much edema and some extreme irritation of the 1.5-inch by 1-inch edematous subcaudal fold. This test was purely informative to show that there was well over 60 to 80 percent infection as determined by these well-marked, three or four X reactions. Dr. A. Karantounias, while visiting at Cornell from the Ministry of Agriculture in Athens, estimated on August 6, 1959, that 15 percent of all cattle in Greece would react to the tuberculin test.

There no doubt were other areas in Greece where there was less tuberculosis. There could not have been areas where there was much more of it. A few cattle had large, hard lumps in the pectoral muscles from vaccination against Johne's disease. This should not, however, cause many false positive tuberculin reactions when mammalian tuberculin is used.

If the authorities in the United States should decide to do something for the cattle owners of the present cattle population of Greece, in addition to sending over good breeding stock for artificial insemination, as they have done since 1946, it would seem that providing replacements of young cattle from areas of the world like the United States where tuberculosis is very, very scarce, would be a good plan. Disinfection of premises where men and cattle live under the same roof in crowded conditions would be possible but not easy. The eradication of bovine tuberculosis there, as here in 1917, looks formidable; but with the use of modern knowledge and plenty of money for replacements and indemnity for the existing diseased cattle the problem of eradication is challenging and no doubt could be accomplished.

It was interesting that in Greece, where tuberculosis was so common, I observed not even a single case of so-called skin tuberculosis. This may be accounted for by the fact that is stressed by the excellent description of this problem disease, which we call acid-fast lymphangitis, by Harry Hedström 1/ in Sweden in 1949. He states: "The fact that the highest frequency has, as a rule, been proved in districts [where there is no tuberculosis] can, according to the author, be due to other circumstances. There are good reasons to suppose that so-called skin tuberculosis, like genuine tuberculosis, is a mycobacteriosis and thus the two diseases may be connected with one another immunilogically. If this is the case, conditions for the outbreak of so-called skin tuberculosis in tuberculous herds would be unfavourable. By analogy to this the frequency of so-called skin tuberculosis would be low in those districts where tuberculosis is common and would increase as the tuberculosis control proceeds."

Traum, 2/ in the original papers on this skin lesion problem, made the same observation; and in a herd that he helped the late D. H. Udall test, in Ithaca in 1922-23, a good reaction to a skin lesion occurred, in a herd where there never had been and never was any tuberculosis.

We should not be discouraged by the fact that skin tuberculosis (so-called acid-fast lymphangitis) and other, unknown causes make a few thousand cows react in clean herds each year in America; but we should leave no stone unturned to see to it that we do not revert to the conditions of 1906-18 when the tuberculin test was new in this country. A good look at conditions in herds such as I have described in Greece would convince any of us that real tuberculosis causes dairy products to be of low value as well as dangerous to the consumer.

1/ Hedström, Harry; Stockholm, 1949: "Studies on So-Called Skin Tuberculosis in Cattle, Concerning Its Prevalence in Sweden. Its Diagnosis, Etiology, and Allergy to Tuberculin." Nord. Vet. Med. 2: 83-99.
2/ Traum, J.; 1946: "..Lymphangitis in Cattle." J.A.V.M.A. 49: 254-257.

VIII. REPLACE OPINIONS WITH FACTS

THE FACTS REGARDING ARS TUBERCULIN--PAST AND PRESENT

J. E. Williams, Laboratory Services, Animal Disease Eradication Division, ARS, USDA, Washington, D.C.

The tuberculin test has been used in detecting tuberculosis in cattle since the early days of the discovery of tuberculin by Robert Koch in 1891. Its accuracy has stood the test of time and usage as one of our most reliable diagnostic reagents in animals. As early as March 31, 1892, tuberculin was employed to test a herd of cattle in Pennsylvania by Dr. Leonard Pearson using tuberculin that he brought from Europe.

The former Bureau of Animal Industry undertook work on tuberculin in 1892, and began to supply the product to State and Federal veterinarians for field use. There was immediate interest in the use of the product for detecting tuberculosis and the demand for it steadily grew. In the year ending June 30, 1906, about one hundred thousand doses were prepared and distributed. During the 12-month period ending June 30, 1959, approximately 10 million doses were prepared and distributed.

Along with the program to produce tuberculin for field distribution, a very active research program involving chemical and biological studies of tuberculins was also undertaken very early, by the former Bureau of Animal Industry. As this research work progressed over a period of many years, the Bureau was recognized as the foremost source of information relating to the production and testing of tuberculins in the United States. Most of the other laboratories working in this field in all parts of the world depended on the Bureau to provide them with standardized materials for checking and improving their own products.

Dr. Marion Dorset, former Chief of the Biochemic Division of the Bureau of Animal Industry, first selected the three human strains of *Mycobacterium tuberculosis* (Pn, C, and DT) that are now used worldwide for the production of tuberculin for both animal and human use. Dr. Dorset isolated these cultures from tuberculous children in Washington, D. C., about 1903. Another significant contribution of the Bureau of Animal Industry was the development of a synthetic medium for the production of tuberculin in 1934. This medium provided a tuberculin of much higher sensitivity and purity as compared with the old tuberculin produced on nonsynthetic mediums. In 1934 the preparation of the old type of tuberculin was discontinued in the United States, and the synthetic medium accepted as the standard. Many other countries adopted the use of this medium as first developed by the Bureau of Animal Industry, and at the present time most tuberculins are produced on the synthetic medium of the ARS.

Cultures are grown on the above medium at a temperature of 37.5° C. for 10 weeks. The average maximum weight of bacteria, which is usually reached before the end of the seventh week, is approximately 2 gm.

(dry weight) per 100 ml. of medium. When the cultures have made their full growth, they will have used up practically all the constituents of the medium. At the end of the growth period, the cultures are sterilized in flowing steam for 3 hours to kill the tubercle bacilli. After being cooled, the sterilized cultures are strained through gauze. The filtrate containing the active principle of tuberculin is evaporated to slightly less than one-fifth of the original volume of the culture medium. Glycerin and phenol are added in equal quantity bringing the total volume to 40 percent of the original volume of the culture medium. Finally the product is filtered and passed through high speed centrifuges until clear and free of all traces of tubercle bacilli.

In 1955 after more than 60 years of experience in preparing various types of tuberculins, the USDA discontinued activity in this field. This action was necessary owing to the lack of adequate facilities for working with tuberculosis cultures. Consequently, a commercial source of the product was developed through contract arrangements under which every stage of tuberculin production is constantly under close supervision by trained ARS personnel. Each step to be followed in production is closely checked and all the procedures used in production are those developed and used by the ARS. Persons associated for many years in the development and production of tuberculin by the Bureau of Animal Industry presently serve as consultants in the production program, and earlier provided the information on which are based our production and testing standards. If at any time questions arise regarding the production of tuberculin obtained from commercial sources, these consultants are available for discussion of the problem; and their recommendations are closely followed. ARS research personnel who have spent many years working in this field are also constantly available to advise us regarding questions that arise in the fields of tuberculin production and evaluation. These practices have insured us a product of highest quality and potency for use in field programs.

In addition to providing detailed specifications on the production of tuberculin, Government supervision includes the use of specific biological and chemical tests both by ARS laboratories at Beltsville, Md., and by the producing firm before the product is released for distribution. ARS biological tests include comparison of the commercially produced product with a previously selected standard produced by ARS, through use of guinea pigs that have been sensitized with cultures of tuberculosis organism. Test results with the commercially produced product must compare very closely with those obtained with the ARS product. All of the data derived from these sensitivity tests in guinea pigs are subjected to statistical analysis, and the final disposition of the product is dependent upon the results obtained. Chemical tests are also conducted to determine the total nitrogen in the product and the nitrogen precipitable by trichloracetic acid. These determinations provide information on the amount of active principle in the product. The acetic acid precipitation test, which has been employed by ARS and the Bureau of Animal Industry for many years for standardizing and checking tuberculins, provides additional information on the amount of active principle in the product.

Chemical tests also include those for the level of phenol in tuberculin and the determination of its hydrogen-ion concentration.

It has been pointed out by those using tuberculin under field conditions that the commercial product is slightly less dark in color than was the ARS product distributed prior to 1955. This difference in color has no relationship to the potency or quality of the tuberculin and is due to the fact that the commercial producer prefers to filter the dextrose solution prior to its addition to the medium. This procedure precludes the heating of the dextrose and results in a lighter color of the finished product, since dextrose upon exposure to heat becomes caramelized and a darker color is formed. It was the practice of ARS in producing tuberculin to add the dextrose to the medium before heating rather than to employ the procedure of filtering the solution before addition to the medium. This matter was thoroughly considered at the time a contract was drawn up for tuberculin production, and it was determined that the addition of the unheated dextrose would in no way alter the potency or quality of the finished tuberculin.

In an effort to accumulate data on the relative sensitivity of ARS-produced tuberculin and that produced under contract, the ARS has undertaken comparative experiments to test the relative sensitivity of the two tuberculins on cattle under field conditions. Herds of cattle for the experimental study were carefully selected on the basis of past history of tuberculosis, and the reactions obtained with the two products were correlated with the findings on post mortem as far as possible. A total of 585 animals on 13 premises in 6 States were represented in the study. Of the total number of animals tested 552 were negative to both tuberculins. Contract tuberculin representative of 8 serials was included in the study. Of a total of 33 animals which reacted to some degree to both tuberculins, in no case did the commercial product detect any reacting animals which were not detected by the ARS product. Neither did the ARS product detect any animals that were not detected by the commercial tuberculin. Reactions to the two tuberculins were quite comparable. Deviations when noted were slight. These comparisons were based on allergic reactions of caudal and vulval tissues.

Dr. Wilder will report on similar comparative studies recently completed in a midwestern State on an epidemiological study.

Results did not indicate more no-gross-lesion (NGL) reactors with commercially produced tuberculin than with the ARS product on the caudal fold test. When suspicious reactors were encountered, the degree of reactions was essentially equal whether ARS or commercially produced tuberculin was used. These results indicate that the various lots of the commercially produced tuberculin and the ARS standard react in a very comparable manner under practical field conditions when used to test cattle. Plans are to extend the comparative tests on a continuing basis to new serial lots of contract tuberculin as it is received by our laboratory.

In an effort to develop more detailed information on factors causing reactions to tuberculin in no-gross-lesion cases under field conditions, the Animal Disease Eradication Division has established an investigational project on tuberculosis at the Ames interim Diagnostic Laboratory and at other laboratories. The program of this unit included detailed laboratory studies of specimens collected from animals revealing NGL reactions. It is hoped that this work will provide additional data on the cause of some of the NGL problems occurring in the field. It is well known that in the field of human tuberculosis many new types of bacterial organisms closely resembling Mycobacterium tuberculosis are being recovered from patients thought to be tubercular. In maintaining the confidence which has been built up in the tuberculin test over a period of many years, it is important to develop newer information on some of the causes of NGL reactions as they are encountered in the field.

The validity of the tuberculin test has been demonstrated by scientists in all parts of the world over a period of many years. Our present tuberculin continues to provide us with one of the most valuable diagnostic agents available to man for the detection of disease. The high standards set for the product in the past will be continued in the future.

GETTING THE FACTS IN PROBLEM HERDS

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Webster indicates a "problem" as something difficult; uncertain; a perplexing situation. Indeed, in dealing with tuberculosis we sometimes come across situations in herds that are "difficult to ascertain"--"uncertain as to outcome"--"perplexing in situation." We indicate these as problem herds.

Let's cite some examples to indicate some of the variations that one is confronted with in problem herd work:

1. Herds in which gross lesions are disclosed on post-mortem examination, and after repeated retests continue to reveal, sporadically, additional gross-lesion reactors.

2. Herds in which reactors removed have disclosed no-gross-lesions and which continue to disclose additional no-gross-lesion reactors on retest.

3. Occasionally herds are found in which some of the animals reveal a very small response to tuberculin, less than is normally classified as a reactor. Such animals have been termed suspects; in other instances they have been called deviators.

On retest of the herd, some responses have remained on retest and are classified as reactors. Sometimes on retest, however, no response is found and the animals are passed as negative.

Of course, one sees from time to time various graduation of these problems. These are just some examples of what you come across when you are dealing with problem herds.

As we have had reported at this conference, there are several known factors that can enter into the problem herd situation. These are the factors known to cause response to tuberculin. They can be categorized as follows:

1. Hypersensitivity due to *Mycobacterium bovis*.
2. Hypersensitivity due to *Mycobacterium avium*.
3. Hypersensitivity due to *Mycobacterium tuberculosis*.
4. Hypersensitivity due to *Mycobacterium paratuberculosis*.
5. Hypersensitivity due to the causitive organism of skin lesions.

These are all known factors causing tuberculin response; however, the exact extent that each of these now enters into all responses occurring in cattle is presently unknown.

Obtaining further information regarding the role of each of these factors is most important to the program at this time. Carefully planned projects to obtain well-documented data that will "stand up" under scrutiny and evaluation are now being carried out.

In discussion and studies of tuberculin hypersensitivity in cattle, one hears of many other opinions or ideas of still other factors as causing tuberculin response. These might here be indicated as a so-called "unknown factor" group and be listed as a sixth category in addition to the five previously mentioned.

The epidemiological projects now underway have been drawn up to bring forth further light regarding any of these so-called "unknown factors" that may enter the problem.

We have discussed so far the realm of the problem herd--the possible factors causing their occurrence--and have indicated that projects are now underway to add further knowledge on them. Let us therefore discuss just what all of us can do right now with regard to our immediate handling of individual problem herd situations. Let us bear in mind that the greater portion of these problem herd situations are readily solved through intelligent application of our present procedures.

The immediate handling of the individual problem herd should be centered around three main aspects:

1. Documentation of the problem in each case is a requirement.
2. Attention and close supervision are necessary.
3. Fullest use must be made of all our present knowledge and tools at hand.

Let us consider point number one, Documentation of the Problem. At this point, in our program, it is extremely important that animals having response to tuberculin in problem herds should be carefully recorded. Such documented evidence will greatly aid in the study of the problem after retests are made. In most cases, responses found in herds are sufficient for reactor classification and removal. However, instances occur where animals with very slight responses are left in the herd pending retest; and, to again repeat, it is very important that these responses be carefully and uniformly recorded along with the individual animal-identification passed tag. Letting such responses go by unrecorded or unclassified lends confusion to a carefully detailed study of a particular situation. Indeed, documentation is a prime issue in getting at the solution.

We mentioned that point number two in dealing with problem herds is Close Supervision and Attention. One sees, from time to time, a situation whereby a problem herd is recognized, but only after the lapse of several retests has the particular problem been given complete attention. Many problem herds develop through lack of attention. On making herd retests, the operator should enter into his work fully apprised with an understanding of the previous herd history. Frequent reviews of all problem herd situations are necessary to assure that complete attention and investigation have been made from all angles.

The last point of stress involving problem herd work is to assure we have done our very best to determine and remove sources of trouble, that is by Making Fullest Use of Our Present Knowledge and Tools. This would involve procedures discussed at this conference to aid in bringing to light any factors contributing to the difficulty. In this regard, four prime aspects should be recognized:

1. Use should be made of the cervical test where circumstances deem it advisable.

2. When conditions involving the herd situation indicate, use should be made of obtaining specimens from reactor animals for submission to the laboratory.

3. Additional close followup should be made as to the thoroughness of the cleaning and disinfection procedures that have taken place. In many instances this facet is overlooked; when these procedures are carefully supervised, the so-called "problem" can, many times, be overcome.

4. The fourth point is that a complete epidemiological investigation should be carried out in connection with every problem herd. The following aspects should, by all means, be investigated and followed up:

a. Herd history on sales or purchases.

b. The health status regarding tuberculosis in other species of animals and poultry at the farm, along with the health history of the human beings involved.

c. Management practices.

d. The farm characteristics--buildings, soil characteristics, drainage problems, barn lots, water systems, and so forth.

The epidemiological investigation should be, as we have stressed earlier, "well documented." Such investigations, when broadly carried out, will certainly further the knowledge regarding the elimination of sources of tuberculosis.

In summary, we have indicated some of the overall problems we face in tuberculosis eradication work today--the factors causing tuberculin response, the extent of the role of each factor unknown at this time. Secondly, the various things we face when dealing with the individual problem herd. The solutions can be gained only by "getting the facts." How is this done?

1. For the overall problem, by obtaining carefully compiled data and information on the various aspects of the problem in <u>sufficient</u> volume so that critical analysis can be made. The word <u>sufficient</u> is important here. A quote from the book "Pathogenesis of Tuberculosis," by Arnold R. Rich, John Hopkins Hospital, would be appropriate:

> "There is little to choose between conclusions drawn from a large number of doubtful histories and those drawn from an insignificant number of accurate histories."

2. For the individual problem herd, may we repeat:

a. Document.
b. Closely supervise.
c. Make full use of our present tools.

FACTS TO BE DERIVED FROM SPECIAL PROJECTS

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During the course of this conference several speakers have pointed out the need for continued field studies and research projects to replace opinion with facts.

My purpose in speaking to you today is to outline the procedures, the mechanics as it were, of special tuberculosis field projects which have been established and which are being developed at selected points throughout the nation, where the number of reactors to the intradermal tuberculin test and the percentage of no-gross-lesion cases are both relatively high.

These special field investigations have a common objective--the accumulation of factual data to answer some of the questions which, in spite of considerable work in years past, still remain essentially in the realm of theory or opinion rather than fact.

It is anticipated that these projects will assist us in gaining additional knowledge regarding the role of avian and human tuberculosis, paratuberculosis, and possibly other factors in the tuberculin testing program.

A cooperative field study of this type was established in Sauk County, Wis., in March of this year (1959). Fieldwork was performed by State and Federal regulatory personnel. The laboratory work, which is still in progress for this project, is being conducted at the Veterinary Science Department of the University of Wisconsin.

The outline of procedure for this field study provides for further evaluation of different dosage levels of tuberculin as well as different types of tuberculins. The procedure also provides for comprehensive epidemiological studies at each farm where reactors were found. Tests on other species of animals were also provided for in the outline of procedure. These included poultry, dogs, cats, swine, sheep, ducks, geese, horses, goats, and mules.

Herds in this study were divided into three separate groups designed as groups A, B, and C. Animals in all groups were injected intradermally in one caudal fold with 0.1 cc. of contract mammalian tuberculin. Animals in group A were injected with 0.1 cc. of ARS tuberculin in the opposite caudal fold. The same procedure was used in B and C, using as inoculants 0.1 cc. of avian tuberculin and 0.05 cc. of contract tuberculin, respectively. The standard 0.1-cc. dose of contract tuberculin was used as the basis for determining reactions. Animals showing tissue responses at the site of injection of P1, X2, or greater were classed as reactors, and tissue responses to other tuberculins were carefully

recorded. A very comprehensive epidemiological checklist was completed at each farm where reactors were found, and all other species of animals on the farm were tuberculin tested.

Reactors to the caudal test were again injected, cervically this time, with the following products:

1. Contract tuberculin
2. ARS tuberculin
3. Avian tuberculin
4. Johnin
5. PPD mammalian
6. PPD avian
7. Control

The first four inoculants were injected in dosages of 0.1 and 0.05 cc., the last three in 0.1-cc. dosage only. The control was made up of phenolized, glycerinated normal saline. These 11 points of injection were carefully measured with a dermal thickness gage prior to and 48 hours after injection. Increases in dermal thickness were carefully measured and recorded.

All lesions that were disclosed, representative lymph nodes, and a section of the intestine including the ileocecal valve, were to be submitted to the laboratory at the University of Wisconsin for histological, bacteriological, and cultural examination. The laboratory work would include the inoculation of laboratory animals with material for purposes of typing the organism.

The completion of the laboratory work, along with the assembly and evaluation of data accumulated in a field study such as this, requires considerably more time than the fieldwork itself. Consequently, the information to be derived from this particular study is only partially available at this time. Considerable work remains to be done in categorizing this information, adapting it to a machine records system, and finally evaluating the data from the fully completed project.

Shortly after the completion of the field testing phase of the special project in Sauk County, it was decided to initiate another field study in Outagamie County, Wis. This project will involve data to be obtained on approximately 40,000 cattle. The project outline will provide for the use of several tuberculins and an evaluation to be made of the double comparative cervical test similar to that being used in England. Laboratory work on specimens obtained from reacting animals will be processed as previously outlined.

A limited study involving 929 cattle in 9 Texas herds was conducted to provide some data on the effects of different dosages of tuberculin. There is need for more extensive and comprehensive studies of this type to determine the optimum diagnostic dose of tuberculin.

Additional cooperative field projects are in the planning stage to provide factual data so essential to the advancement and success of the tuberculosis eradication program.

IX. COMPREHENSIVE DIAGNOSIS

PROCEDURES OF THE MEAT INSPECTION DIVISION IN EXAMINATION OF REACTOR ANIMALS

Leslie J. Rafoth, Chief Staff Officer for Animal Foods, Meat Inspection Division, ARS, USDA, Washington, D. C.

I am very happy to take part in this Tuberculosis Conference to explain the part meat inspection has in the eradication of tuberculosis. This afternoon we will explain and demonstrate post-mortem inspection procedures used on tuberculin reactors.

Cattle reacting to the tuberculin test are received at establishments operating under Federal Meat Inspection as U.S. Suspects. They are penned separately from other animals and slaughtered at a time specified by our veterinarians in charge of cattle slaughtering operations and post-mortem inspection. The slaughter time is determined by the workload of the inspectors and the facilities available for performing the examinations.

Reactors are identified by U.S. Retained tags throughout all phases of inspection. When the head is removed from the animal, 5-section retained tags are used on the carcass and head so absolute identification can be maintained. When the viscera are removed from the carcass, one of the retained tags identifies these tissues.

Post-mortem inspection of reactors is divided into three phases--head, viscera, and carcass inspection. Lymph nodes examined during head inspection are those associated with tissue considered as primary locations of infection. Because the lymph nodes of the head are associated with the upper respiratory and digestive systems and both may be primary seats of infection, more lymph nodes of the head are found to be affected with lesions than other tissues. Viscera inspection consists of an examination of lymph nodes receiving lymph from the lungs, stomachs, intestines, and liver as well as the examination of these organs and the spleen. Most lesions found during examination of the viscera are in the bronchial and mediastinal lymph nodes associated with the respiratory system and the mesenteric nodes associated with the digestive system. Both locations are considered primary seats of infection. Lung tissue lesions are considered primary lesions and are often found along with bronchial and mediastinal node pathology. Lesions in the spleen and liver are generally secondary and are usually found only when primary lesions are present. During inspection of reactors, the spleen, liver, and hepatic lymph nodes are examined even though lesions are not found in primary seats of infection. This precludes the possibility of overlooking lesions that happen to be atypical. Carcass inspection consists of an examination of body lymph nodes, body cavities, and kidneys. Infection of the carcass lymph nodes is usually considered secondary, but in the case of tuberculin reactors these nodes are examined to determine if exposure might have been the result of injury and introduction of *Mycobacterium tuberculosis*.

Reactors are identified on post-mortem reports from the ear tags and not from information copied from forms TE-27. All ear tags are held in field meat inspection offices for 6 months after reporting. During my assignment in the field, occasional TE-27's showed numbers of the listed reactor tags transposed. In such cases, the actual reactor tag number is reported and the transposition of numbers is called to the attention of the ADE officials if our inspector notices the discrepancy at the time of reporting.

Tagging of brucellosis reactors with tuberculosis reactor tags increases inspectional demands at the slaughterhouse. When veterinary meat inspectors find animals identified with tuberculosis reactor tags and no brands, they perform a tuberculin reactor post-mortem examination which is more extensive than the routine inspection given brucellosis reactors. If the improper tagging is reversed and tuberculin reactors are identified with brucellosis reactor tags and the animal is not branded or poorly branded, the usual tuberculin reactor post mortem will not be given such an animal.

After September 1 a new form for reporting lesions and dispositions of tuberculin reactors will be used by our post-mortem inspectors. I would like to explain a few facts about it that might be of interest to you:

1. One form will be used for each reactor.

2. Results will be tabulated by machine in Chicago.

3. Results obtained from the use of this form may make it possible to modify our procedure some time in the future.

4. The post-mortem report for any reactor will be readily available at all times.

5. It will be possible to determine the pattern of infection associated with infection in specific lymph nodes.

Many cases of tuberculosis are found during regular post-mortem inspection. Identification of such affected animals is often made through brands, ear tags, selling price, name of commission company, breed of animal, whether horned or dehorned, barn tags, physical deformities, and dressed weight--so our post-mortem inspectors are instructed to furnish you people with any or all of this known data. The procedure used during our regular examination of cattle is responsible for the number of tuberculous nonreactor animals found. Regular post-mortem inspection entails examination by slicing or palpation of all major lymph nodes associated with tissues where primary infection can occur.

COLLECTION OF SPECIMENS FOR LABORATORY DIAGNOSIS OF TUBERCULOSIS AND PARATUBERCULOSIS

Wayne D. Yoder, Diagnostic Laboratory, Animal Disease Eradication Division, ARS, USDA, Ames, Iowa

In order to assure accurate diagnosis, the collection of specimens must be carried out in such a manner as to assure no contamination. Also, the specimens must be collected with considerable precision to prevent excessive slowdown of the operations at the packing plant. Only specimens from herds giving considerable trouble should be submitted. This is made necessary by the limited facilities at the laboratory.

Prior to the testing of the problem herd a shipping container should be ordered from the laboratory. The shipping container is an insulated chest that will hold specimens from two or three animals.

In addition to the shipping container the following materials will be needed;

1. Formalin
2. 70-percent ethyl alcohol
3. Sterile cotton
4. Scalpels
5. Forceps
6. Specimen bottles
7. Identification tags or stickers for the specimen bottles
8. Carbon dioxide ice
9. Pan for disinfectant

The formalin, alcohol, and sterile cotton can be purchased at a drugstore. The formalin as purchased contains about 38-percent formaldehyde. To obtain a satisfactory formalin solution, 1 part of the formalin, as purchased, should be diluted with 9 parts of water.

The scalpels, forceps, and specimen bottles can be obtained commercially. The scalpels, foreceps, and specimen bottles must be sterilized before they are used for the collection of specimens. This can be done by dry heat (150 C. for 2 hours) or by boiling in water.

If specimens from more than one animal are to be obtained, at least three scalpels and three forceps will be needed. This is necessary so that a sterile set can be used on each animal from which specimens are obtained. Thus, two sets can be sterilized in 70-percent alcohol while the other set is being used.

The tags used for identification of specimen bottles can be marking tags with string attached. A portion of the string can be placed in the tissue bottle and then secured in place by screwing the cap down. The tags should be marked with the reactor tag number of the animal and the anatomical region from which the tissue came.

Tissues from each animal should be collected at the following locations:

1. Lymph nodes of the head.
2. Lymph nodes of the thorax.
3. Lymph nodes of the abdomen.
4. Lymph nodes of the body, including the supramammary.

This does not mean all nodes, but portions of representative nodes of each region. As each node is removed, a portion of it is placed in a sterile bottle and another portion in a bottle filled about three-fourths full with formalin. It should be remembered that the bottles containing formalin should not be filled with tissue. There should be an excess of formalin remaining. As soon as the tissues for bacteriological examination are collected, they should be placed in the insulated box with the CO_2 ice and frozen. The formalized tissues should <u>never</u> be frozen. They should be packed separately and mailed to the laboratory.

The frozen tissues should be shipped to the laboratory air express. This means that the air schedules for express must be checked to make sure that the tissues arrive at the laboratory within 36 hours after shipping. The laboratory should be notified as to the exact time of the arrival of the tissues at the Des Moines Airport. The box should be labeled as follows:

Dr. M. J. Eggert
A.D.E. Diagnostic Laboratory
Veterinary Quadrangle
Ames, Iowa

<u>Hold at Airport in Des Moines</u>

Each of the two boxes containing tissues should also include the following:

1. Name and address of the shipper.
2. Name and address of the owner of the animals from which tissues are submitted.
3. Disease suspected.
4. Complete history of herd.

The box containing the tissues in formalin can be shipped by parcel post.

The foregoing has dealt mainly with tuberculosis. In paratuberculosis, or Johne's disease, small sections of intestine, particularly near the ileocecal valve, and including all of the layers of the intestine, should be sent. It would be well to include a few mesenteric lymph nodes. These specimens would be mailed air express under refrigeration. A similar group of tissues (intestine and lymph nodes) should be formalized as described previously and mailed parcel post.

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